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EXPERIMENTAL STUDIES CONCERNING THE SITE OF ORIGIN OF BILIRUBIN

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The question whether, under normal conditions, bilirubin is formed within the liver cells or somewhere outside of them has provoked theoretical discussion and experimental study for many years. The final solution of this problem must, quite naturally, form the basis for our more accurate knowledge of the physiology and pathology of the bile pigments. Indeed, when one turns to the study of the various and little understood forms of jaundice which occur in the absence of any mechanical obstruction to the flow of bile through the bile-passages, the very crux of the situation lies in the question: Where is the pigment being formed? Only through this knowledge shall we ever be able to understand the mechanism of the icterus in these diseases.

To everyone who has given any attention to the matter it is well known that we are no longer able to say glibly that the hepatic cell is the site of origin of bilirubin, and with that to let the question rest. Indeed, it is fair to say that no satisfactory proof has ever been brought forward to show that the liver cell is actually able to produce this pigment. It has been customary to point to the occurrence of bilirubin within the tumor cells of primary carcinoma of the liver as evidence that the hepatic cell itself can elaborate the pigment, but it must be clear that this phenomenon could be explained equally well on the view that the liver cell takes up bilirubin from the blood stream as a part of its excretory function. That the liver cell does actually take up bile pigment from the circulation has been clearly established by Wertheimer.[15] The experiments of Whipple and Hooper,[16] which seemed to indicate that bilirubin production could be increased by carbohydrate feeding, constituted

perhaps the most suggestive evidence that the liver cell itself could synthesize the pigment; however, the recent researches of Rous, Brown and McMaster [17] have demonstrated that, because of the experimental methods used by Whipple and Hooper, such a conclusion is entirely unwarranted. There is no other evidence of any significance pointing to the activity of the liver cells in bilirubin production. On the other hand, fairly satisfactory evidence has been accumulated to prove that bilirubin can be formed outside the liver cell. The most convincing observation in this regard is Virchow's well-known discovery of the local formation of "hematoidin" in blood extravasations.[1] This has been repeatedly confirmed by many investigators, who have been able to demonstrate the appearance of the pigment in practically all parts of the body where red blood cells have escaped from the blood vessels and have disintegrated. Although the chemical proof that "hematoidin" and bilirubin are identical is still wanting, and certain thoughtful writers on the subject are willing to go no further than to say that "hematoidin" resembles bilirubin very closely,[2,18] still, since analysis shows that both substances have the same composition althongh the structure of neither is known, and since "hematoidin" exhibits chemical and physical reactions characteristic of bilirubin, and since it arises from the same molecule (hemoglobin), which we know can yield bilirubin, most writers have regarded the pigment formed at the site of blood extravasation as bilirubin itself.

Although the question of the formation of bilirubin in localized blood extravasations is a very important one indeed, and merits more careful study not only with regard to the chemical nature of the pigment formed but also the type of cell which can effect the transformation, it concerns only indirectly the question of the origin of the bilirubin which is normally present in quite constant concentration in human blood plasma; and it is not clearly related to the origin of the high bilirubin content of the blood in the non-obstructive jaundices in which abnormal blood extravasation plays no rôle. In these diseases one wonders (1) whether the liver cells themselves are producing the bilirubin and are pouring an excess of it into the blood stream instead of into the bile canaliculi, or (2) whether bilirubin is being made somewhere outside the liver cells, is discharged into the blood stream and accumulates there either because of a disturbance of a normal excretory function of the liver or because the pigment is being produced in such abnormal amounts that the liver is unable to excrete all that is brought to it by the circulation. These are the possibilities which have formed the skeletons of the many "hepatogenous" and "anhepatogenous" theories of jaundice; and practically all of the theories stand today as possibilities simply because we do not yet know what cells are able to produce bilirubin. I shall not attempt here to review the enormous literature dealing with the origin of bilirubin, for there are too many aspects which do not bear at all upon the experiments which form the purpose of this particular paper. But I must recall briefly three researches which have been generally regarded as the most important contributions to the problem of whether hemoglobin introduced into the blood stream can be transformed into circulating bilirubin without the intervention of the liver.

In 1886 Minkowski and Naunyn [3] reported a series of experiments which were at once widely accepted as the proof that there could be no formation of bilirubin from circulating hemoglobin without the participation of the liver. They attacked the problem by the direct procedure of studying the fate of circulating hemoglobin after extirpation of the liver. In mammals the arrangement of the portal circulation is a serious obstacle to the extirpation of the organ; but the circulatory arrangement of birds is such that the liver can be removed without interfering with the venous outflow from the gastrointestinal tract. Minkowski and Naunyn therefore turned their attention to the study of experimental jaundice in ducks and geese. Briefly, they found that if normal animals were forced to inhale arseniureted hydrogen a severe intravascular hemolysis occurred which was rapidly followed by the appearance of a large amount of bile pigment in the urine. If, on the other hand, hepatectomized ducks or geese were subjected to the same treatment, just as severe an hemolysis occurred (as was evident from the intense hemoglobinuria), but the urine either remained entirely free from bile pigment or else contained only minimal ("höchst minimal") amounts, which the authors attributed to the fact that in no experiment were they able to remove the entire liver, and further, to the circumstance that the operation necessitated a leakage of bile from the cut liver into the abdominal cavity, which could be resorbed and so appear in the urine; and finally, the resorption of the pigment from the intestine direct into the blood stream was regarded as probable. Certainly, the possibility of resorption of bile from the peritoneal cavity must be given serious consideration in any experiment during which the liver is cut into and any fragment, however small, left behind; and when we read that in one of the experiments the liver was merely crushed and left *in situ*, the probability of such resorption of bile looms large indeed.

In spite of the importance of the question and the fact that Minkowski and Naunyn had reported experiments upon only a few animals, astonishingly widespread credence was immediately accorded their conclusion that the liver is necessary for the conversion of circulating hemoglobin into bile pigment, and no important reexamination of the matter was undertaken until twenty-seven years later, when McNee,[4] working in Aschoff's laboratory, carefully repeated the experiments with re-

sults which were not fundamentally different from those of Minkowski and Naunyn but which were interpreted in a rather different manner. McNee found, in contrast to the intense jaundice produced by arseniureted hydrogen in normal geese in which not only the urine but also the blood became rich in bile pigment, that either no bile pigment at all or else only minimal amounts appeared in the urine of hepatectomized geese subjected to the poison, and the pigment was never detected in the blood. To explain this slight appearance of pigment after removal of the liver McNee stressed even more than Minkowski and Naunyn the fact that a small piece of liver, because of its proximity to the vena cava, was always unavoidably left within the animal's body, and that microscopic examination of this liver tissue at the end of the experiment showed that it was not necrotic. He felt that the probability of the continued functioning of this tissue had to be considered, and indeed, our own study of the blood supply to this particular region of the liver strengthens this thought considerably. However, McNee's conclusion, based, doubtless, upon the earlier work of Löwit [20] and others, was that no marked jaundice occurred after removal of the liver not merely because the liver cells proper had been removed from the body, but rather because the Kupffer cells contained in the liver had been removed. He had noted phagocytosis of red blood cells by the Kupffer cells in normal animals, and further the presence of iron pigment in these cells. During the hemolytic jaundice following inhalation of arseniureted hydrogen, the presence of phagocytized red corpuscles and iron pigment within the Kupffer cells was much more marked; and he concluded that within these cells hemoglobin is broken down into iron pigment and an iron-free residue which may be bile pigment itself or else an intermediate product in the synthesis of bile pigment, a conclusion formulated in Löwit's splendid paper twenty-four years earlier. Since similar cells could be found in much smaller numbers in the spleen and bone-marrow, McNee thought that perhaps the activity of these phagocytic cells in the latter organs might be responsible for a part of the small amount of bile pigment found in the urine after liver extirpation. In general, however, McNee's conclusion was that the number of such cells outside the liver was inconsiderable in geese and that in hepatectomized birds bile-pigment could never be formed in the spleen and bone-marrow in quantities large enough to be detected in the blood. It should be borne in mind that the possibility of resorption of bile from the peritoneal cavity is quite as important a consideration in McNee's experiments as in those of Minkowski and Naunyn.

The fact that the experiments of these investigators had been carried out upon birds was for many reasons always a source of objection whenever the attempt was made to apply the results to higher animals. It remained for Whipple and Hooper [5] to extend the experiments to mammals, with results which were opposed to the conclusions of Minkowski and Naunyn and which have seemed to furnish a clear proof that the liver is *not* necessary for the formation of bile pigment from circulating hemoglobin. Whipple and Hooper, working independently at the same time as McNee (1913), reported that the change from circulating hemoglobin into bilirubin will take place as promptly in mammals (dog) from which the liver has been removed from the circulation as in normal controls. The methods by which they sought to exclude the liver from the circulation were (1) the production of an Eck fistula combined with ligation of the hepatic artery and (2) the establishment of a "head-thorax circulation" which was attained by ligation of the aorta just below the subclavian artery and again at the level of and including the coeliac axis, ligation of the inferior vena cava above the diaphragm, of the mammary vessels in the thorax and by the further precaution of twisting wire ligatures about the abdominal muscles along the costal margins. In such an animal the liver and all organs below the diaphragm were apparently entirely cut off from the blood stream which then circulated through the head and thorax only. When laked red blood corpuscles were now introduced into the circulation by way of the jugular vein, Whipple and Hooper found that the blood plasma, which at the beginning of the experiment was entirely free from bilirubin, gave a positive test for this pigment as early as two hours after the introduction of the hemoglobin (laked cells). Indeed, at the end of this period of time not only did the plasma contain bile pigment but an actual tissue icterus of the thorax was visible and bilirubin could be demonstrated chemically in the subcutaneous fat. The authors believed that the pigment was probably formed by the vascular endothelium. For ten years these experiments have been widely regarded in all countries [6,7,8,9] as an irrefutable proof that the liver is not necessary for the prompt transformation of circulating hemoglobin into bilirubin, even by investigators who have not been able to reconcile some of their own observations with such a view.

At the beginning of a study of a certain phase of non-obstructive jaundice it was found that, in spite of its vascularity, the spleen took no part in the production of bilirubin from circulating hemoglobin in experimental hemolytic icterus,[19] and it became desirable to re-examine the question of extra-hepatic bilirubin production. Certainly the experiments of Whipple and Hooper were carefully enough carried out and clear-cut enough in result; but the liver was left within the body during their observations and the possibility of the persistence of an effective collateral circulation, although at the time an apparently remote possibility, arose nevertheless to disturb the acceptance of their conclusions as final. And

so it was decided not merely to tie the liver off from all obvious vascular connections, but actually to extirpate the organ and then to study the fate of hemoglobin introduced into the blood stream, in order to put at rest any uncertainty as to the possible participation of the liver in Whipple and Hooper's experiments.

Method

Full-grown healthy dogs were used in all of the experiments to be described.

At the outset it was found to be a quite difficult matter to keep the animals alive in good condition for several hours after extirpation of the liver. This difficulty has long been recognized, and indeed McNee states in his article that "dogs cannot survive the extirpation of the liver for more than half an hour." Many operative methods were tried; but I shall outline here only the routine procedure which was finally found to be the most satisfactory and by means of which a series of eight dogs have been kept under observation in good condition from one to six hours after complete removal of the liver.

The animal is anesthetized with ether and bled from the jugular vein into citrate; the blood is centrifugalized and the supernatant plasma is tested for bilirubin in order to be sure that the dog does not enter the experiment with a bilirubinaemia, for dog's plasma never contains bilirubin normally. The corpuscles are washed in normal salt solution, recentrifugalized and 25 c.c. of the packed red cells laked with distilled water are used for the injection. During the preparation of the cells, the abdomen is opened along the midline and the coeliac axis, the abdominal aorta just above it, the mesenteric arteries, the renal vessels and the inferior vena cava just above the renal veins are successively ligated. The blood vessels and bile-ducts at the hilum of the liver are cut between ligatures and the liver freed from all peritoneal connections except the reflection about the vena cava where this vessel passes through the diaphragm above the liver. Then the oesophagus is ligated at the cardia, a clamp placed below the ligature, the tissue between clamp and ligature divided and the stomach, together with all of the intestines, the spleen and the pancreas, quickly removed from the body in one mass. Both kidneys are then removed to exclude any possibility of the escape of even a trace of bilirubin through the urine. A clamp is next placed across the vena cava between the liver and the diaphragm and a strong ligature is tied tightly above the clamp. Then the vena cava is severed above and below the liver, separating the organ from its last connections with the body so that it can be entirely removed together with the strip of vena cava attached to it, after which the clamp is removed from the ligated stump at the diaphragm. In some experiments the removal of the liver was facilitated by tying the vena cava above the diaphragm through a small inter-costal or diaphragmatic incision under artificial respiration, after which the liver can be more safely cut away, since neither hemorrhage from the vena cava nor air embolism is then to be feared. Bleeding from the diaphragmatic stump is easily controlled by means of a purse-string suture. During the entire procedure, which can be completed within half an hour, the liver is handled as gently as possible in order to avoid squeezing bile into the blood stream, and at no time is the organ cut into or lacerated in any way. Warm towels are put into the abdominal cavity, one placed so as to occupy the site of the liver in order to prevent downward ballooning of the diaphragm. The abdomen is then closed, and after a sample of blood has been taken for bilirubin tests, the laked red cells are introduced into the circulation through the external jugular vein, after which the ether is suspended and the animal kept quiet under morphia during the remainder of the experiment.

In most cases in which the operation proceeds with reasonable swiftness and without loss of blood, the animals remain in good condition up to the point of death; the mucous membranes and tongue are pink, the respiration and pulse are regular and the eye reflex continually active. Death results from sudden cardiac failure. An animal that had appeared to be in splendid condition a few moments before will be found gasping for breath because of an abrupt cessation of the heart beat. Artificial respiration is of no avail whatever at this point. Indeed, a similar cardiac death occurs in animals kept continually under artificial respiration. The intravenous administration of glucose is a very potent means of prolonging the life of the hepatectomized dogs. However, during these experiments an effort was made to introduce into the circulation no more fluid than necessary, in order not to dilute the plasma and so render the detection of small amounts of bilirubin difficult.

Under these conditions, the blood of hepatectomized dogs was studied every half hour after the intravenous injection of laked red blood cells, in order to determine whether any of the circulating hemoglobin could be converted into bilirubin in the absence of the liver. In every case, from the moment after the injection of the laked cells until the end of the experiment, the carefully withdrawn centrifugalized serum was cherry red in color because of the free hemoglobin present. The sensitive Van den Bergh test for bilirubin [10] was used on the 10 c.c. samples of blood taken every half hour. When this test was rendered difficult, as is sometimes the case in the presence of excessive hemolysis, it was supplemented by a modification of it which will be described in another place. In addition to the half-hour tests, the Huppert-Salkowski test, as applied by Whipple and Hooper,[5] was always carried out upon much larger amounts of blood taken from the heart and great vessels of the thorax immediately at the end of the experiment.

Control tests were carried out each time upon other sera known to contain small amounts of bilirubin in the presence of free hemoglobin, in order to be certain that all test solutions were active. Subcutaneous fat was tested for bilirubin by the method described by Whipple and Hooper.[5]

Is Bilirubin Formed From Hemoglobin Circulating Outside the Liver?

In a series of eight experiments carried out exactly as outlined above, and in which the animals survived in apparently splendid condition from one hour and ten minutes to six hours after removal of the liver and from one to five and a half hours after the introduction of hemoglobin (laked cells) into the circulation, not a trace of bilirubin was ever detected by either the Van den Bergh or the Huppert test in any sample of blood taken either during or at the end of the experiments. In no case was there the slightest tingeing of the fat tissue with bilirubin such as that described by Whipple and Hooper, and chemical tests for the pigment made upon the fat tissue were consistently negative. The following protocol of the animal which survived longest is typical of the procedures and findings in all the other experiments:—

Extirpation of Liver. Injection of Hemoglobin. No Formation of Bilirubin.

Dog 28. Mongrel, female, weight 13 pounds. February 15, 1923. Dog is in splendid condition. 10.45 a.m. ether anesthesia; bled from jugular vein into citrate. Plasma colorless and 30 c.c. give negative test for bilirubin. 10.55 a.m. given 1/8 gr. morphia and operation begun. Ligation of coeliac axis and aorta at that level. Ligation of mesenteric arteries, of renal vessels, and of vena cava below liver. All structures at hilum of liver cut between mass ligatures and liver freed from peritoneal connections. Ligation of oesophagus just below diaphragm. Removal of stomach, intestines, spleen, pancreas and kidneys. Artificial respiration established and inferior vena cava ligated in thorax through small incision in diaphragm. Liver cut free from diaphragm and removed intact from the body. Purse-string suture placed about diaphragmatic stump to control bleeding from cut diaphragmatic vessels. Abdomen packed with warm towels and closed. Operation completed at 11.30 a.m. Animal in good condition. 11.40 a.m. given 1/8 gr. morphia and 25 c.c. laked packed red cells injected into jugular vein followed by 25 c.c. 1% glucose. 12.20 a.m., 10 c.c. of blood taken for test. 12.30 p.m., given 40 c.c. 1% glucose intravenously. Animal in splendid condition. 12.50 p.m., 10 c.c. of blood taken; given 1/8 gr. morphia. 1.20 p.m., 10 c.c. of blood taken. 1.25 p.m. given 40 c.c. of 1% glucose intravenously. Condition excellent; tongue pink; eye reflex very active; heart-beat strong. 1.50 p.m., 10 c.c. of blood taken; given 1/8 gr. of morphia. 2.20 p.m., 10 c.c. of blood taken; given 40 c.c. of 1% glucose intravenously. 2.50 p.m., 10 c.c. of blood taken; struggles during manipulation of cannula; given 1/8 gr. of morphia. Condition excellent; heart-beat strong and regular. 3.20 p.m., 10 c.c. of blood taken; given 40 c.c. of 1% glucose intravenously. 3.50 p.m., given 1/8 gr. of morphia; 10 c.c. of blood taken. 4.20 p. m., 10 c.c. of blood taken; given 40 c.c. of 1% glucose intravenously. 4.50 p.m., 10 c.c. of blood taken; given 1/8 gr. of morphia. 5.20 p.m., 10 c.c. of blood taken; heart-beat regular and strong; tongue pink; eye reflex active; compressed air used for artificial respiration cut off throughout the building, so dog was killed by bleeding, although still in splendid condition.

The serum after centrifugalization is claret red in color. The Huppert test applied to 35 c.c. of this serum is negative for bilirubin. The Van den Bergh test on each of the half-hour specimens of serum, all of which are deep red in color, is in every instance entirely negative for bilirubin. The same test solutions are applied to a control serum known to contain small amounts of bilirubin as well as free hemoglobin, and the color reactions are sharply positive in each case.

Autopsy.—Performed at once. The subcutaneous fat appears normally pale in color. The epicardial fat is normal in appearance. Subcutaneous fat tissue (8 grams) from the thorax is negative for bile pigment. The heart and lungs are normal. The ligatures on the vena cava and aorta occlude these vessels completely. There has been a slight leakage of blood from the diaphragmatic incision and a small clot representing perhaps 10 c.c. of blood is found in the abdominal cavity.

In this experiment, which is typical of all, hemoglobin in amount large enough to give to the serum the color of claret remained in active circulation in a dog without a liver for five and a half hours, during which time not a trace of it was transformed into bilirubin. A table summarizing the experiments is given below:

Liver extirpation followed by intravenous injection of laked cells

Dog	Weight	Survival time after injection of laked cells	Van den Bergh test for bilirubin in serum q. ½ hr.	Huppert test for bilirubin in serum at end of experiment
11	13¾ lbs.	1 hour	Negative	Negative
13	17 lbs.	1 hour	Negative	Negative
27	15¼ lbs.	1 hour	Negative	Negative
15	14 lbs.	1 hr. 20 min.	Negative	Negative
12	9 lbs.	1 hr. 35 min.	Negative	Negative
14	19 lbs.	1 hr. 40 min.	Negative	Negative
16	12¾ lbs.	2 hrs. 10 min.	Negative	Negative
28	13 lbs.	5 hrs. 30 min.	Negative	Negative

These experiments are not at all in accord with the widely accepted conclusions of Whipple and Hooper concerning the relation of the liver to bilirubin production. It will be recalled that those investigators found that the serum of their dogs, with liver and all abdominal organs supposedly cut off from the circulation, gave a positive test for bilirubin as early as one hour and fifty-five minutes after the injection of laked red cells; indeed, that enough hemoglobin had been transformed into bilirubin in that brief period of time to bring about a definite, visible bile-staining of the subcutaneous fat, and that bilirubin could be demonstrated chemically in this fat. Whipple and Hooper made no effort to determine the earliest moment at which bilirubin could be detected in the blood following the injection of laked cells into the head-thorax circulation; but if bilirubin were produced within one hour and fifty-five minutes in a quantity great enough to cause a visible tissue jaundice, traces

of the pigment were very probably present in the blood considerably before that lapse of time, so that my animals that lived even an hour or an hour and a half could have been expected to show at least a minimal bilirubinaemia. It is to be remembered that, owing to the total absence of bilirubin from normal dog's plasma, this pigment can easily be detected whenever present in very minute traces; for the detection of the first appearance of the pigment is not a matter of small quantitative differences in amount—it is a matter of no bilirubin at all or the presence of a trace of it, and the tests permit detection of the pigment in concentrations as minute as one to one and a half million.[10] However, even in my hepatectomized animals which lived two hours and ten minutes and five hours and a half (nearly three times as long as the survival time of Whipple and Hooper's dogs presenting an actual tissue jaundice) not a trace of hemoglobin had been converted into bilirubin.

Does the So-Called "Head-Thorax Circulation" Really Exclude the Liver from the Circulation?

The question at once arose—was the liver in Whipple and Hooper's experiments actually cut off from the circulation? These authors state that, after ligation of the portal vein and abdominal aorta at the coeliac axis, "with a clamp upon the aorta below the subclavian artery, there can be no active circulation through any of the diaphragmatic and ligamentary collaterals to the liver."[14] However, in order to test this very important point, I have performed the simple experiment of injecting India ink into the jugular vein in a series of six dogs after having tied securely every blood vessel ligatured by Whipple and Hooper in their head-thorax circulation experiments, i. e., the aorta just below the subclavian artery, the inferior vena cava just above the diaphragm, the right and left mammary arteries and veins within the thorax, the coeliac axis and the abdominal aorta at that level, and, besides, all of the vessels entering the hilum of the liver. Wire ligatures were also placed about the rectus muscles and along the costal margin. In a few moments the ink, propelled by the heart's force alone, became distinctly visible to the naked eye in the lobules of the liver. At autopsy all ligatures were intact, and in every case completely occluded the lumina of the various vessels. It was immediately clear that the liver had not been cut off from the blood stream by the head-thorax circulation method used in Whipple and Hooper's experiments.

It is indeed a remarkable and unexpected thing to see with what ease and promptness the ink finds its way into the portions of the aorta and mammary arteries which lie beyond the ligatures, so that the aorta, for example, is filled with ink throughout its entire extent in spite of being ligated at the two sites described above, and the intercostal and diaphragmatic arteries stand out as black lines. The fact that the ink enters the liver, although the portal vein and hepatic artery are ligated at the hilum, demonstrates that the communication of the liver with the circulation in the head-thorax method is through the diaphragmatic vessels which enter the substance of the liver in the region of the hepatic veins. These vessels are by no means inconsiderable in size and number. But three further experiments were carried out in which, in addition to Whipple and Hooper's ligatures, the oesophagus was ligated and cut across just below the diaphragm and the stomach and intestines, spleen and pancreas removed *in toto,* so that the liver was left attached by its connection with the diaphragm alone. In two experiments, ink injected into the aorta just beyond the aortic valves promptly appeared in the liver, showing beyond question that it could have entered only through the diaphragmatic vessels. In the third experiment the cannula was placed in the aorta below the subclavian artery. The diaphragm promptly became black with ink which quickly appeared in the liver, demonstrating that the phrenic artery is not a necessary pathway for the circulation through the diaphragm to the liver. There is an abundant arterial supply to the diaphragm from the lower intercostal arteries. This is of interest because certain investigators studying the circulation of the liver have quite overlooked this source of blood supply, declaring that the diaphragmatic collaterals to the liver must be negligible because of the small size of the phrenic artery.

In order to determine whether, in spite of the ligature on the vena cava, there were venous return channels from the liver to the heart as efficient as the arterial paths to the organ, the portal vein was cannulated in two experiments and India ink allowed to run in from a burette after we had ligated, above the diaphragm, the vena cava, the aorta and the mammary arteries and veins. The liver was quickly blackened with ink, of course, and in a few moments the diaphragmatic vessels were injected and the lungs became quite black, showing that there was an adequate venous return from the liver through the diaphragmatic vessels, although the vena cava was securely tied two centimeters above the diaphragm.

I think that anyone who takes the time to repeat these simple procedures cannot fail to be struck with the ease with which blood can find its way into and out of the liver after the tying of ligatures which for years have been regarded as sufficient to exclude the liver from the circulation. Some years ago I made use of the head-thorax circulation method in the attempt to exclude the liver from the blood stream during the course of a study of certain phases of blood coagulation,[11] and it may be of interest here that I was surprised to find in the blood of the head-thorax animal quite as prompt an appearance of the substance which I was studying and which had previously been shown to be formed in the liver, as

in normal animals—an occasion apparently quite comparable to Whipple and Hooper's finding as rapid an appearance of bilirubin in the serum of head-thorax dogs as in the normal. Very recently Pickering and Hewett have used the same method in a study of blood coagulation with results which have led them to deny the existence of antithrombin. It seems clear that their work, as well as that of all investigators who have made use of the "head-thorax circulation" method, may be open to criticism in the light of the present experiments.

The vascular connections between the liver and the diaphragm vary considerably in size and richness in different dogs, and this may explain, perhaps, the different results which have been found to follow complete ligation of the hepatic artery. It has been the experience of a number of investigators that in some dogs such a procedure is followed by a widespread necrosis of the liver cells; in others no such necrosis occurs. Indeed, Haberer, whose work upon the effects of ligation of the hepatic artery in its various portions is regarded as classic, has stated that he found large diaphragmatic collaterals in all cases in which necrosis failed to follow complete ligation of the hepatic artery.* [12] An examination of Whipple and Hooper's protocols shows that, of their series of five "head-thorax circulation" experiments, in only three could bilirubin be demonstrated definitely in the serum and body fat at the end of the experiment. In a fourth case the serum gave only a "very faint" and the fat a "positive suspicious" test for the pigment; while in the last case the serum and fat tests were not positive. It seems quite possible that these last two experiments may have been carried out upon dogs in which the diaphragmatic blood supply to the liver was very small. As for Whipple and Hooper's positive Eck-fistula hepatic artery ligation experiments, it is worthy of note that in three out of six such experiments the authors state that the ligation of the blood vessels at the hilum of the liver was found at autopsy to be incomplete; so that in addition to the probability of the liver receiving sufficient blood from the diaphragmatic vessels there was the certainty of the persistence of an infra-diaphragmatic blood supply in half of the experiments.

* Although in most of these experiments the ink found its way into all parts of the liver, even to the very edge, the area of the organ within a radius of several centimeters about the vena cava just below the diaphragm, is, of course, the most readily supplied with blood from the diaphragmatic vessels. Other investigators have noted that one cannot kill this portion of the liver by ligating the portal vein and the hepatic artery. I have mentioned that McNee was aware of this; Minkowski and Naunyn had observed it before him, and Stern, whose work [21] antedates Minkowski and Naunyn's, clearly drew attention to the same fact in 1885.

Can the Liver Continue to Function When Supplied Only by the Diaphragmatic Vessels?

The India-ink experiments just described demonstrate conclusively that fluid introduced into the jugular vein of a "head-thorax circulation" animal enters the liver by way of the diaphragmatic vessels, can drive out and replace the blood from the lobules and return again to the heart. This appears to be clear enough evidence that the laked blood introduced in Whipple and Hooper's experiments must also have circulated through the liver. Since, as the present experiments show, no bilirubin is formed from circulating laked blood when the liver has been actually extirpated, one must believe that the diaphragmatic supply to the liver was sufficient to permit it to carry on its function of changing hemoglobin into bilirubin during the brief period of Whipple and Hooper's experiments, even though this amount of blood must represent only a small fraction of the normal supply to the organ. In view of the extraordinary vascular anastomoses within the liver, one cannot lightly assume that the organ cannot continue to live when its blood supply is greatly reduced. We are familiar with the fact that, when the entire portal blood stream is shut off from the liver (Eck fistula), the organ continues to function indefinitely, although its blood supply is suddenly and enormously reduced. Recently, Dr. Schlaepfer of the Surgical Department has shown me microscopical preparations from his experiments upon dogs demonstrating that the lung which, like the liver, is an organ of double blood supply and rich vascular anastomoses, can withstand the sudden ligation of the entire pulmonary artery over a period of days without death of the tissue, its nutrition being maintained only by the relatively insignificant bronchial arteries. One knows that organs such as the liver and lungs, which have the function of regulating the chemical composition of the entire blood, receive vastly more blood than is necessary for their nutrition.

I have made no attempt so far to test the function of the hepatic cells under the conditions of the "head-thorax circulation," but I have been able to demonstrate that the Kupffer cells (which perhaps may yet be proven to be the most important part of the liver as far as bilirubin production is concerned) remain functionally active under these conditions. These cells are well known to possess marked phagocytic properties. In three experiments the "head-thorax circulation" was established strictly according to the method of Whipple and Hooper described above and the animal allowed to lie quietly for half an hour. Then a small quantity of India ink (1 c.c. 50% ink pro kilo.) was injected into the jugular vein, and after an hour the animal was killed, an autopsy performed to be certain that all ligatures were intact, and pieces of liver taken for microscopic

study. Macroscopically, the ink was clearly visible in the liver, and in the sections the Kupffer cells stood out strikingly because they were filled with phagocytized ink particles, demonstrating that they remain alive and continue to perform their phagocytic function in spite of the fact that they are nourished only by blood from the diaphragmatic arteries. Figures 1 and 2 are microphotographs of the liver from one of these dogs.

Total Exclusion of the Liver from the Circulation without Extirpation

It would be unreasonable to believe that the complete absence of bilirubin production in the blood stream of the dogs without livers could be ascribed to the fact that they had undergone a more severe operation than Whipple and Hooper's dogs and were therefor perhaps in poorer condition; for Whipple and Hooper's dogs dying spontaneously about two and a half hours after the operation can hardly be thought to have been in a better condition than, for instance, the dog of my series which had to be killed six hours after removal of the liver. I emphasize again that up to the point of death in each experiment the heart beat of my dogs was strong, the circulation active enough to keep the mucous membranes pink in color, and the eye reflex active. But even such a possible objection has been satisfactorily controlled by the exclusion of the liver from the circulation with ligatures. When we learned that in the head-thorax method of Whipple and Hooper the blood reaches the liver through the diaphragmatic vessels, it became evident that if we interrupt the vascular connections between the liver and diaphragm after ligating the portal vein and the hepatic artery, no blood can possibly find its way to the liver. This experiment has been performed upon five dogs. The procedure in each case was exactly as that given in the following protocol:

Dog 32, male, mongrel, weight 14 pounds. March 28, 1923. Dog in excellent condition. 10.30 a.m. ether anesthesia and bled from jugular vein into citrate. Centrifugalized plasma colorless and gives negative test for bile pigment. Abdomen opened along midline; aorta ligated at coeliac axis; coeliac axis and mesenteric arteries ligated; portal vein and hepatic artery ligated at hilum of liver. Ligature tied about vena cava between liver and diaphragm; *this ligature ineludes all vessels connecting liver and diaphragm since these vessels run along the vena cava.* Vena cava tied again just above renal veins. Operation finished at 10.50 a.m. At 10.55 a.m. injection of 25 c.c. laked packed red cells into jugular vein. Given ¼ gr. morphia. Ether suspended; animal in splendid condition. At 11.25 a.m. given 50 c.c. 10% glucose intravenously and 1/8 gr. morphia subcutaneously. Condition excellent. At 12 N. given 1/8 gr. morphia. Heart beat strong; respiration regular; eye reflex very active; tongue pink. At 2.15 p.m. animal in splendid condition; killed by bleeding. Thorax opened at once. Aorta cannulated just above aortic valves and India ink injected; diaphragm becomes intensely black and the arteries are filled with ink up to the point of the ligature between the liver and diaphragm, but no trace of ink is found anywhere in the liver, either macroscopically or microscopically, showing that the liver was entirely cut off from the circulation. At autopsy all ligatures are intact and subcutaneous fat is normal in color.

The serum after centrifugalization is cherry-red in color. The Van den Bergh test for bilirubin is clearly negative. 35 c.c. give negative Huppert test for bilirubin. Subcutaneous fat (16 grams) also gives negative test for bilirubin. Sharp positive reaction is obtained when the same test solutions are applied to serum known to contain a small quantity of bilirubin, as well as a large amount of free hemoglobin.

This experiment shows first that the liver can be excluded from the circulation by means of proper ligatures, the important point being the tying of the vena cava just *below* the diaphragm instead of just *above* it as has been formerly done. In the second place, the experiment answers any objection which might be raised against the extirpation experiments on the ground of the severity of the operation; for the operation used in this experiment is much simpler and much less severe than that used by Whipple and Hooper which involves ligation of the aorta, vena cava and mammary vessels within the thorax. And yet here again, with the liver excluded from the blood stream, not a trace of circulating hemoglobin is converted into bilirubin in experiments lasting over three hours.* A summary of these ligation experiments is given here.

Liver tied off from circulation. Injection of hemoglobin. No bilirubin found.

Dog No	Weight	Survival time after injection of hemoglobin	Bilirubin tests on serum at end of experiment	Bilirubin test on subcutaneous fat
32	14 lbs.	3 hrs. 20 min.	Negative	Negative
33	16 lbs.	1 hr. 30 min.	Negative	Negative
30	17 lbs.	2 hrs. 5 min.	Negative	Negative
34	15 lbs.	2 hrs. 15 min.	Negative	Negative
40	14¾ lbs.	3 hrs. 15 min.	Negative	Negative

Discussion

As to the significance of the present experiments, we feel that it becomes necessary to abandon the belief that there exists a facile and ready mechanism for the transformation of circulating hemoglobin into bilirubin outside the liver, and to return once again to the old view that the transformation takes place normally only within the liver itself. Whether certain cells outside the liver (in the spleen and bone-marrow?) can gradually take over the function of bilirubin production, in pathological states where a compensatory reaction is called for, must remain unanswered here. But this, if it does occur, must require the persistence of a need over a period of time or some factor other than the mere presence of free hemoglobin in the blood stream coupled with the sudden absence of the liver.

* I must emphasize the necessity for a control injection of India ink into the aorta after every experiment in which this method of liver exclusion is used, in order to determine whether all ligatures were efficient. The region of the liver about the diaphragmatic ligature should be examined with especial care.

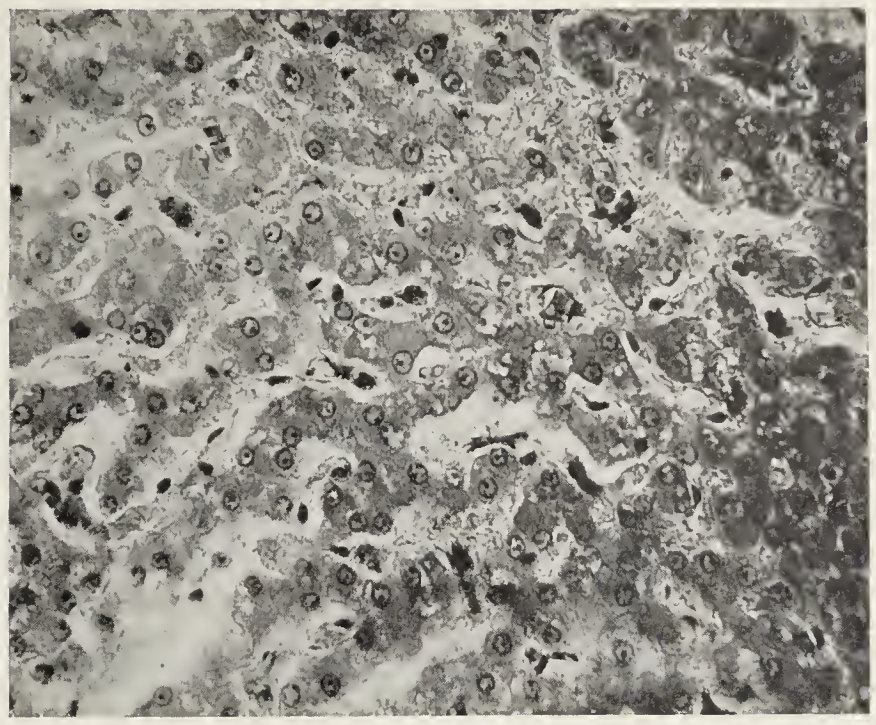

Fig. 1.—Is a low power microphotograph showing that India ink passes readily from the heart into the liver after the establishment of the so-called "head-thorax circulation" and that the Kupffer cells retain their phagocytic function under these conditions.

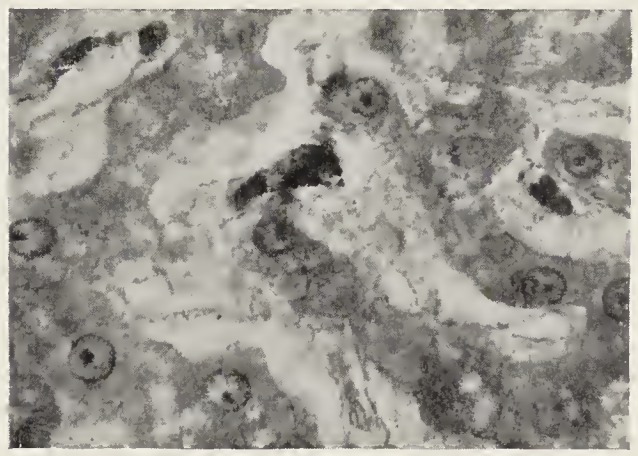

Fig. 2.—Is a high power microphotograph of one of the Kupffer cells to show the phagocytosis of the ink. See text for details of this experiment.

I do not mean to imply that this constitutes any evidence whatever that the *hepatic* cell is capable of producing bilirubin. On the contrary, the sole function of the liver cells in regard to bilirubin may be an excretory one, and it may be the Kupffer cells which possess the function of splitting the hemoglobin molecule into bilirubin and an iron residue. If one turns to the early work of Minkowski and Naunyn,[3] one finds beautiful pictures which show with all clearness the presence in the Kupffer cells of red cell fragments, iron pigment and a green pigment which the authors unhesitatingly believed to be bile pigment produced within these cells from the hemoglobin of the ingested red blood corpuscles ("Hiernach kann es kein Zweifel sein, dass der Blutfarbstoff in den Blutkörperchenhaltigen Zellen zu Gallenfarbstoff zerstezt wird"). Although Minkowski and Naunyn did not recognize them as such, the illustrations accompanying their paper leave no doubt whatever that the "Blutkörperchenhaltigen" cells were the Kupffer cells of the liver. Since that observation the thought has arisen often in the minds of many investigators that the Kupffer cell may be the site of origin of bilirubin. It will be remembered that this was the kernel of McNee's conclusions. In recent years Eppinger[13] has sought to create a place in pathology for the "reticulo-endothelium," and has been perhaps the most insistent advocate of the view that bilirubin production is a function of the Kupffer cell. So far, however, no conclusive evidence has been brought forward to establish this belief as a fact, and, in spite of the tempting advantages of such a view, we must at present believe only that normally bilirubin is produced somewhere within the liver, and seek to determine just what part may be played in its production by the vascular (Kupffer) cells on the one hand and by the parenchymatous cells on the other.

It will be noted that the experiments reported in this paper are concerned with the fate of *circulating* hemoglobin, and the conclusions are applicable only to hemoglobin which is circulating in the blood stream, not to hemoglobin liberated into the tissues from extravasated blood. The conversion of hemorrhage hemoglobin into bilirubin (hematoidin) is, of course, a purely local phenomenon, the exact mechanism of which still remains a question for study.

Conclusions

1. Hemoglobin introduced into the blood stream of a dog without a liver, and circulating actively as long as five and a half hours, is not transformed into bilirubin. Since, however, the production of bilirubin from circulating hemoglobin takes place readily and rapidly in a dog with a liver through which the blood stream passes, it is concluded that the liver is necessary for the transformation of circulating hemoglobin into bilirubin.

2. Experiments which have led to the conclusion that circulating hemoglobin can be rapidly transformed into bilirubin outside the liver do not prove that conclusion, because the "head-thorax circulation" method which has been used in such experiments does not actually exclude the liver from the circulation.

3. A method for the exclusion of the liver from the circulation without removing it from the body is described.

I wish to express my gratitude to Dr. W. G. MacCallum for his helpful criticism.

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A CLINICAL STUDY OF ACUTE STREPTOCOCCUS INFECTION OF THE PHARYNGEAL LYMPHOID TISSUE (ACUTE FOLLICULAR TONSILLITIS)*

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In a series of investigations carried out during the fall and winter of 1922-23, certain observations have been presented bearing on the etiology, epidemiology, and prevention of acute streptococcus infections, with special reference to acute tonsillitis.[1] It has seemed desirable to make a clinical analytical study of acute tonsillitis as seen among a group of healthy young adults (nurses and house physicians of the Johns Hopkins Hospital). Members of such a group offered special advantages for clinical investigation in that they were invariably seen early in the course of the infection, and that all cases were under close observation in the hospital wards throughout the period of illness and convalescence.

Definition

Acute tonsillitis is an acute infectious disease of the lymph adenoid tissue of the oropharynx, caused by the hemolytic streptococcus, having a characteristic symptom-complex, and a rather typical course, lasting usually from three to five days.

Etiology

The Cause.—The specific micro-organism causing the disease is *Streptococcus hemolyticus* of the beta type as described by Brown.[2] With the employment of Brown's poured-plate technique, practically pure cultures of beta-hemolytic streptococcus have been obtained in one hundred per cent of the cases during the acute stage of the infection. It seems apparent from the experimental evidence at hand [3] that, although the organisms isolated from a large series of tonsillitis cases are not identical in all their biological and cultural characteristics, they are members of very intimately related races of beta-hemolytic streptococci.

It seems to us of fundamental importance that this fact should be better appreciated, in order that the disease itself, fixed on a firm etiological basis, may take its place with erysipelas, for example, as a definite specific hemolytic streptococcus infection, rather than as a mere clinical syndrome which follows in the train of any bacterial infection of the pharynx. It is to be stressed the more because of the statement widely spread throughout practically all text-books of medicine and stomatology that acute tonsillitis has a varied etiology—the streptococcus, staphylococcus albus and aureus, and pneumoccus being included as agents (Packard,[4] Breitstein,[5] Ballenger,[6] and Stevens [7]). MacCallum [8] regarded the streptococcus viridans as a cause of tonsillitis, although it is now definitely established that the green streptococci represent, with the Gram-negative cocci, the fixed normal flora of the mouth (Bloomfield [9]). As recently as the present year, Barnes [10] has stated that, although the streptococcus pyogenes is the infecting organism in a large percentage of cases, the streptococcus viridans, the pneumococcus, staphylococci, and rarely the colon bacillus, are occasionally responsible.

We are inclined to believe that this misconception has come about through the employment of surface cultures instead of the poured "shake" cultures as used especially by Brown. If both methods are employed simultaneously in studying the throat flora of a tonsillitis case, the reason for variations in interpretation instantly becomes obvious. The mere spreading of a throat culture on the surface of a blood-agar plate allows the unrepressed growth of the larger colonies, which then tend to overshadow the more delicate and slower growing colonies of the hemolytic pathogen. Embedded in the depth of the agar, all organisms are subjected more or less to the same influences, and overgrowth of one organism by another is impossible. Further, the phenomenon of hemolysis can be accurately studied only on poured plates, and the classification of alpha and beta types of hemolytic streptococci is based on this technical procedure.

In short, the present investigation has led us to believe that in no case of acute tonsillitis have organisms other than the beta-hemolytic streptococcus been found to play a pathogenic rôle.

Predisposing factors.—Although it is a current belief, expressed in many text-books (Kyle,[11] Barnes,[10] Osler,[12] Stevens [7]), that exposure to cold or wet weather is the frequent antecedent of and predisposing factor in the onset of acute tonsillitis, special consideration of this point in taking histories has failed to demonstrate any etiological bearing. Studies of general meteorological conditions, presented in a previous paper (Hodges [13]) of the present series, have similarly failed to attach any etiological importance to weather variations.

However, certain facts seem to have been well established as predisposing influences (Bloomfield and Felty [14]). In contrast to the oft-made statement that tonsillitis is

* This is the fourth of a series of papers on streptococcus infections with special reference to acute tonsillitis.

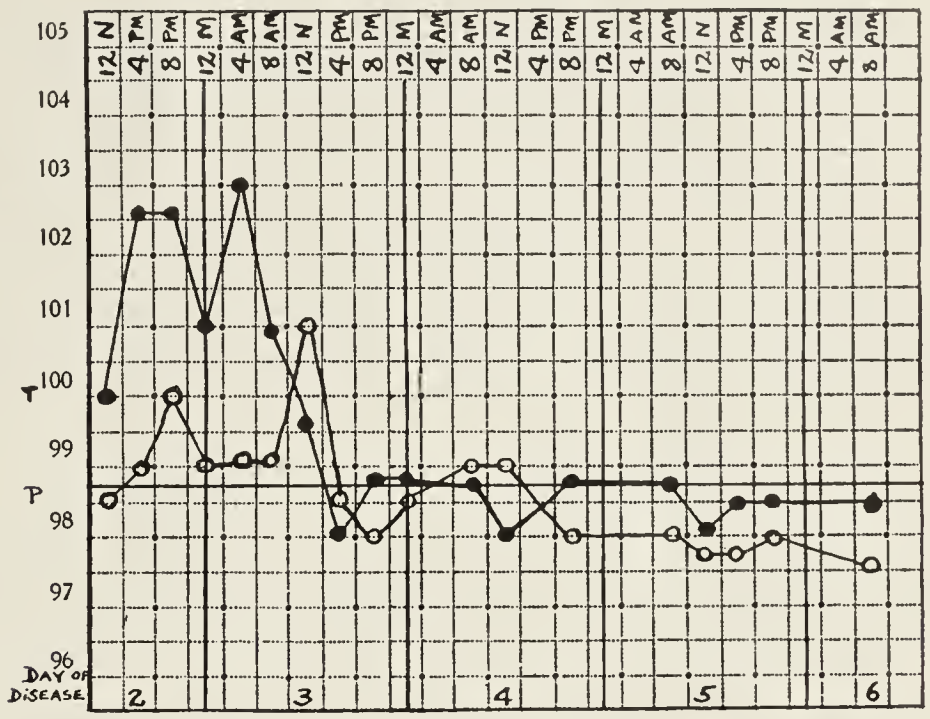
due to auto-infection (Barnes[10] among others believes that the infecting organism is, in many cases at least, one of those chronically present in the crypt of the tonsil), it has been demonstrated that a state of focal tonsillar carriage of the hemolytic streptococcus, usually the residuum of a previous attack, either produces or is evidence of a high degree of immunity. Tonsillectomy confers relative insusceptibility to hemolytic streptococcus infection, although, as will be indicated later, acute disease of the lymphadenoid tissue of the posterior pharynx, clinically and etiologically identical with acute tonsillitis, is of not infrequent occurrence. Certain individuals seem to possess a considerable degree of natural immunity to this type of infection, as has been noted in the case of other infectious diseases; and conversely, other individuals of peculiar susceptibility are the victims of repeated attacks.

The chronic carrier of the hemolytic streptococcus, shown by many observers to constitute a large percentage of the community (Bloomfield and Felty[1]), is the obvious focus of infection from which new cases develop, as in the spread of typhoid fever, etc. However, intimate and probably prolonged contact such as would be brought about by crowding is apparently essential for the active localization and growth of the microbic agent in the lymphadenoid tissues (Bloomfield and Felty[15]). Such conditions of prolonged and intimate contact commonly obtain only in the cold seasons of the year when the activities of the community are largely carried on indoors. This fact offers a reasonable explanation of the prevalence of the disease during the winter months, and its almost complete disappearance during the summer.

Clinical Picture

Emphasis has already been placed on the invariable presence of a single type of microbic agent in all cases of acute tonsillitis thus far studied. In keeping with this unity of etiology one finds a rather characteristic clinical picture, with a more or less constant onset, symptomatology, physical findings, course, and termination. Before considering these points in detail, it may be of interest to describe a typical uncomplicated case of acute tonsillitis of moderate severity.

Following a period of good health or of mild indisposition due to a slight coryza, the individual suddenly notices, often on awakening in the morning, a moderate soreness and fullness of the throat with discomfort on swallowing. Associated with this there is usually a headache, often frontal, and a sense of malaise—a fatigability which may make him cease his activities. During the next twenty-four hours the patient feels feverish and has chilly sensations and the angina and dysphagia become more severe. The malaise increases during the first day, the back and extremities may ache, and the patient is sufficiently prostrated to go to bed. The appetite is poor; the patient may have slight nausea, though he does not vomit. The voice is thick from the edema of the pharynx, though hoarseness from laryngitis is uncommon. When seen after twenty-four hours, the patient shows slight prostration, and appears frankly ill. There is a faint flushing of the face, and perhaps also a slight erythema over the chest. The anterior cervical lymph nodes, especially those located just beneath the angles of the jaw are swollen, and tender; the posterior cervical glands may also be mildly inflamed. The patient may resist movement of the head on account of the tenderness in the neck. The eyes perhaps show some conjunctiv-



Temperature chart of typical mild case of acute tonsillitis

al injection. The throat itself presents a perfectly characteristic picture. From the anterior faucial pillars to the posterior pharyngeal wall it is diffusely reddened and inflamed. The uvula too is edematous and hyperemic. The tongue is slightly coated; the breath is fetid. All the lymphadenoid structures of the oropharynx—including the tonsils and those less conglomerate collections of adenoid tissue behind the pillars on the posterior pharyngeal wall—are much swollen and intensely injected. The surface of the tonsils is spotted with pin-head-sized points of yellowish white, purulent exudate, coming from the crypts, which may be so profuse that it may appear as free pus discharging into the buccal cavity. Examination of the chest and abdomen is generally negative.

The temperature within twenty-four hours reaches 101° to 103°F. with coincident sweating and chilliness, the pulse is quickened to 110-120, and the patient presents all the features of an acute infectious process. The angina and dysphagia are distressing, and the malaise, associated with restlessness and wakefulness, continues. The typical "urine of fever" is present—of high specific gravity and deep color, with perhaps slight albuminuria. These constitutional symptoms continue for another twenty-four hours during which period the temperature

is irregularly elevated from 100° to 103° or higher. On the third or fourth day the temperature takes a critical drop, falling from its peak of 102°, or above, to normal within the course of from twelve to eighteen hours. A slight secondary rise for a few hours may follow, though characteristically the temperature remains normal following the crisis, and throughout the convalescence. Coincident with the critical fall in temperature, there is generally a symptomatic crisis, with a rapid disappearance of the constitutional symptoms—the malaise, general aching, prostration, and headache—and the patient, while still a little weak, feels greatly relieved. The angina and associated dysphagia and the tenderness of the submandibular glands clear up more slowly. After two or three days the exudate on the tonsils disappears, the edema of the pharyngeal structures subsides, though the throat may remain injected for a week or more after the active symptoms have ceased. Convalescence is rapid and there is never the residual asthenia so common after influenzal attacks. The patient is well enough to resume his activities generally within a week after the onset of the disease.

Analysis of Forty Cases of Acute Tonsillitis

Age and Sex.—These factors can not be considered in view of the fact that the group studied did not represent a cross-section of the average community.

Season.—Reference is made to a preceding paper of this series in which the consideration of seasonal incidence is taken up in detail.[13]

Symptomatology, Onset.—In practically every case, the onset is acute, symptoms appearing in the midst of good health. In a few of the cases, coryza preceded the acute onset.

General Symptoms.—Except in the case which is so mild that the patient does not come under observation, the constitutional reaction is rather typical and uniform. Synchronously with the rapid development of the angina, there is a sense of ill-being and weakness, and the patient has chilly sensations alternating with feverishness. In about fifteen per cent of the cases, there was a history of one or more attacks of frank rigor. Marked sweating is also common. All of these constitutional symptoms appear occasionally before the angina develops, and in such cases, especially when there is aching in the back and extremities, the clinical picture may for the moment suggest influenza.

Pharynx.—As a rule, the first symptom noted is soreness of the throat with pain on swallowing, either alone or more generally associated with general constitutional symptoms, which develop simultaneously with the angina. The local symptoms usually reach their height in twenty-four to forty-eight hours, and gradually clear up, the soreness lasting longer than the malaise. The pharynx presents a uniform appearance in nearly all cases—general edema and hyperemia of all the structures including the uvula, marked swelling of the tonsils and sometimes of the lymphoid tissue of the posterior pharyngeal wall, often with prominence of the follicles, and with small discrete spots of yellow, gray, or white exudate over the surfaces of both. The exudate can be removed and is found to contain detritus, white cells, and streptococci. In severe cases free pus is present in the posterior oral cavity. In a few cases the exudate is confluent and membranous, and there may be crater-like, punched-out areas of necrosis on one or both tonsils, not unlike that seen in Vincent's angina. The "tight, full feeling" so often complained of may or may not be associated with excessive local edema.

Glands.—In almost all cases, even the mild ones, there is local cervical glandular enlargement, with soreness, and tenderness on palpation. The gland just beneath the angle of the mandible on either side, in the anterior cervical chain, commonly referred to as the "tonsillar gland," is always affected. Less commonly the posterior cervical and lower anterior cervical nodes may be swollen and tender. This swelling and tenderness usually persist after the constitutional symptoms are over, clearing with the improvement of the pharynx.

Head.—Headache occurs as a common and early symptom. It is usually a dull pain, but sometimes severe and throbbing. Except when it is due to a local infection in a sinus, it usually disappears with the constitutional symptoms. Sinusitis has not been observed early in the disease, and rhinitis with nasal discharge coming on with the sore throat was present in but three cases. Nasal epistaxis was the initial symptom in one case. In a few instances, patients complain of photophobia and lacrimation and in about one fourth of the cases, there was conjunctival injection of a greater or less degree. In no cases were there symptoms referable to the ears, and aside from slight hyperemia of the blood vessels observed in two cases, the drums were normal.

Cardio-respiratory.—Cough is an uncommon symptom and hoarseness with laryngitis was present in but one case. The respirations are not increased. The lungs were clear in all cases. Tachycardia is the rule at the onset (100-140), the pulse generally falling with the temperature. We have not observed the bradycardia which has been described in cases of septic sore throat.[16]

Gastro-intestinal.—As part of the general constitutional reaction, gastro-intestinal symptoms often are present shortly after the onset. Anorexia is common, and nausea is complained of in about one third of the observed cases, although vomiting is much less frequent. Diarrhea initiated the attack in one case. The tongue is usually coated. Abdominal examination reveals little. In no case was the spleen demonstrably enlarged.

Skin.—The skin manifestations are slight. In about one half of the cases there was an erythema of the face.

In six cases, this erythema was present also on the chest or was even more extensively distributed—in the axillæ, on the back, abdomen, and arms. Although the rash was not typically scarlatinoid, but rather an erythematous or a discrete or confluent maculopapular eruption, in one case it was difficult to differentiate the condition from atypical scarlet fever. Herpes was present in none of the cases.

Blood.—The red cells and hemoglobin are not affected in this disease. The usual leucocytic reaction characteristic of streptococcus infections is present, the white count during the first forty-eight hours averaging approximately 16,000. The leucocytes varied from 8,500 in a mild abortive case to 32,000 which was the highest count observed. In all cases there was a relative polynucleosis of from 75%-90%. A moderate leucocytosis persists in the uncomplicated case for four to seven days after the complete disappearance of fever and of local and general symptoms. The degree of leucocytosis seems to bear no definite relation to the severity of the symptoms, fever, or course of the disease.

Urine.—On admission the urine is generally concentrated and of high color, since most patients on account of the dysphagia do not of their own accord take fluids freely. In one fourth of the cases the urine showed a trace of albumin; in but one case was there frank albuminuria with casts, which subsequently disappeared.

Course and Termination

Among the uncomplicated cases of the series studied, the duration of fever and general symptoms varied from one to seven days, with an average duration of three days. The total period of disability (from the onset of the general symptoms until discharge from the hospital) was from four to fifteen days, with an average of seven days, and the average period of hospitalization was six days. The maximal temperatures observed in the hospital ranged from 99.5° in a mild case, to 105° in a case characterized by a severe constitutional reaction. The average maximal temperature was 102°F.

The characteristic termination of the fever is by a sharp critical fall to normal within eighteen hours. This phenomenon occurred five times as frequently as the slower subsidence of fever. In nearly all cases the general symptoms of malaise disappeared coincidently with the fall in temperature, though the throat and cervical glands remained sore for from one to three days after the crisis.

Abortive and Atypical Cases

Many cases of acute tonsillitis are so mild as to be ambulatory, and thus fail to come under careful observation. The constitutional symptoms may be very slight and transitory, or absent, and the only complaint may be the soreness of the throat. Fever, if present, is usually slight and lasts only twenty-four hours. The pharynx presents a picture similar to but less intense than that seen in the severer cases. The throat culture always shows the specific organism in predominance.

It has been noted above that tonsillectomy does not always confer immunity from subsequent hemolytic streptococcus infections of the pharynx. This is, of course, due to the presence of other susceptible masses of lymphoid tissue behind the posterior faucial pillars remaining after removal of the tonsils. When these clumps of adenoid tissue become infected, a train of events follows which clinically resembles tonsillitis, and the bacteriological picture is identical. In four out of five such cases observed the past winter the course was somewhat less severe than that of the average case of true tonsillitis.

Reference has already been made to those cases simulating scarlet fever.

Prognosis

In the uncomplicated case, the prognosis is invariably favorable. Thus, the outcome depends entirely on the nature of any complications which may arise. Since general systemic infections do not usually occur as in epidemic septic sore throat, the outlook is the more favorable, though the occasional mastoid or sinus infection following tonsillitis demands immediate attention. No cases of death due to complication occurred in our series.

Complications

The occurrence of complications is the exception in the course of this infection, although further involvement of proximal structures is occasionally seen. However, the organism does not appear to have the invasive power that is seen in the milk-borne epidemic form of sore throat, and localization in distant organs, *e. g.*, the peritoneum, and joints, has not been observed.

Eight, or twenty per cent, of the forty cases studied showed some form of complication. Of these eight patients, four had acute infections of one or more paranasal sinuses, two developed peritonsillar abscess, one had acute otitis media with mastoiditis, and one a transient bacteriemia. Where cultures were made, the organism causing the complication was found to be the hemolytic streptococcus, except in the one case of blood-stream infection, in which a streptococcus viridans was recovered.

In all but two cases, there was an interval of normal temperature of twelve hours to four days between the critical termination of the primary disease and the first manifestation of the complication. In one case of quinsy and one case of sinusitis the fever did not fall characteristically on account of the supervening extension of the infection. In all instances, the complications received prompt consideration by the laryngologist and except for the one case of streptococcus mastoiditis which required major surgical interference and a prolonged

convalescence, the average period of disability and hospitalization was increased but one week.

The one case of systemic infection is of interest. After an afebrile, asymptomatic period of two days, the patient had a sharp chill and fever of 103°, and prostration. Quantitative blood culture showed less than one colony per cubic centimeter of a green streptococcus. In twenty-four hours the temperature fell from 103° to normal by crisis, with general improvement which thereafter continued during a short uneventful convalescence. The blood was sterile on subsequent cultures.

No cases of renal complications such as occur in scarlet fever have been seen.

Differential Diagnosis

Simple Pharyngitis.—This affection is usually a feature of the common "cold," associated with rhinitis and tracheitis. In addition to the mildness of the symptoms and the absence of any exudate or of acute lymphadenoid inflammation in the pharynx, the flora varies little from that found in the healthy throat. The bacteriological study may serve as a differential point between pharyngitis and mild tonsillitis.

Vincent's angina.—Clinically this may simulate mild acute tonsillitis, and the differentiation depends entirely on the finding of the fuso-spirillary forms diagnostic of the disease.

Diphtheria.—As in the case of Vincent's angina, the differential diagnosis is essentially a bacteriological one, since certain cases of diphtheria are at first clinically indistinguishable from acute tonsillitis. Further, a diphtheria carrier may develop tonsillitis, and culture on Loeffler's medium alone may confuse rather than aid in diagnosis. It is, therefore, desirable to culture all cases of severe angina on both Loeffler's medium and on poured blood agar plates.

Epidemic Septic Sore Throat.—Whereas in the differential diagnosis of the above conditions the study of the throat flora is of first importance, in the case of epidemic septic sore throat, cultures of the pharynx yield in one hundred per cent of the cases hemolytic streptococci in predominance, exactly as is found in acute tonsillitis. Hence bacteriological study affords no aid in distinguishing between the two conditions, which must be differentiated on the basis of the history, epidemiological data, and on the course of the disease. Indeed, it is by no means certain that the two diseases are basically different—on the one hand, a hemolytic streptococcus gains a foothold in the adenoid tissue of the throat by transfer from a carrier or an active case; on the other, the organisms, probably in a highly vegetative, actively growing state in a favorable medium (milk), and in huge numbers, constitute a massive inoculum on the susceptible pharyngeal tissue. This difference in vegetative activity and numerical dosage of the bacteria, as well as possible inherent pathogenicity may in itself play a great part in altering the clinical aspects, the severity, and the prognosis of these two diseases of essentially identical etiology. Davis[17] says that it may be impossible to distinguish between the individual case of epidemic septic sore throat and severe sporadic cases of acute tonsillitis. However, the explosive outburst of such an epidemic, associated quite definitely with an infected milk supply, and characterized in general by much severer symptoms and the tendency to serious complications, are features which in most cases serve to differentiate the milk-borne infection from the more common acute tonsillitis.

Scarlet Fever.—Scarlet fever is essentially a clinical syndrome, and the diagnosis depends entirely on the fairly characteristic history and the typical rash, angina, and other usual findings. Bacteriological culture of the inflamed pharynx presents a picture quite indistinguishable from that found in acute tonsillitis, i. e., an overwhelming predominance of beta-hemolytic streptococci. The recent investigations of Tunnicliff,[18] Gordon,[19] and Bliss,[20] have thrown a new light on the whole subject by showing that the beta-hemolytic streptococci found in scarlatinal angina form a distinct immunological group sharply delimited by serological methods from streptococci isolated from other infections. The extreme technical difficulties of obtaining suitable bacterial suspensions and of making and maintaining immune serum of high titer quite preclude at present the introduction of such a differential test as a routine laboratory procedure.

The outspoken case of scarlet fever, on the one hand, and the frank acute tonsillitis case on the other, present clinically no diagnostic difficulties. Between these two extremes, there are occasional cases with atypical rashes of irregular distribution, with a pharyngeal lymphoid inflammation resembling follicular tonsillitis, with an absence of hypertrophic lingual papillae, and without the subsequent development of nephritis—these cases test the critique of the most expert diagnostician. Indeed it would seem that in such instances the clinical evidence at hand is entirely inadequate to distinguish between atypical scarlet fever and tonsillitis with a concurrent streptococcus rash, and the ultimate differentiation falls on the serologist who is able to identify only by most refined methods the streptococcus of scarlatinal angina from the less specific streptococcus of tonsillitis. The significance of these border-line cases is so great that it demands the reopening of the now old controversy on the etiology of scarlet fever, and justifies the attitude of many observers that a streptococcus hemolyticus is the actual cause of the disease rather than a concomitant invader.

Therapy

Acute tonsillitis, like other streptococcus infections (*e. g.* erysipelas) is a self-limited disease of short duration, and once established cannot be aborted or its course measurably shortened. There is no specific therapy available. Hence the treatment is purely palliative, and effort is directed solely toward symptomatic relief.

General Measures.—If the attack is of any severity, the patient should be and probably will choose to be confined to bed. In spite of the dysphagia, fluids should be forced, as in any acute infection, up to three or four liters a day. Food is of secondary consideration since the nutrition will not suffer during the brief attack. Any liquid or bland soft diet is adequate. The bowels should, of course, be moved freely. Among the many analgesics and sedatives in use, Dover's powder, with codein and phenacetin or acetylsalicylic acid seem to give the greatest relief. Since the actual duration of general discomfort is short, it has been our custom to administer immediately maximal doses of all three drugs, i. e., Dover's powder gr.x (0.65 gm.) with codein gr. i. (65 mgm.) and phenacetin gr. x (0.65 gm.). The patient experiences prompt relief and is enabled to drop off to sleep.

Local Measures.—The use of an ice collar is often desirable, especially where the adenitis is severe. Feeding cracked ice is also of value. We feel that the use of so-called antiseptics such as argyrol, the mercurochromes, or of escharotics like a solution of silver nitrate is to be condemned. The infection is not superficial, but penetrates well into the lymphoid tissue, and such local applications cannot do more than destroy surface bacteria. Furthermore, there is a definite theoretical objection to such practice. The adenoid tissue is already acutely inflamed, and should not be subjected to further insults in the form of strong antiseptics and caustics, or even traumatized through abrasion. In fact, the less trauma done to the inflamed tissues, the better. However, simple gargles, used hot, while having little effect on the local process improve oral hygiene. So, too, throat irrigations of hot normal salt solution (114°F.) from a gravity bottle give great symptomatic relief.

Prophylaxis.—The attendants should take the usual precautions observed in handling infectious patients, particularly the wearing of masks, since there is little doubt that the spread of the disease takes place through droplet infection.

Preventive Therapy.—From a small series of observations,[21] it appears likely that vaccination with killed cultures of the hemolytic streptococcus confers relative immunity.

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SICKLE CELL ANAEMIA*

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Introduction

Reference to sickle cell anaemia has appeared only four times in the literature. The first mention of the disease is found in a case report by Herrick [1] in 1910. A second case was reported by Washburn [2] in 1911 and a third by Cook and Myer [3] in 1915. The fourth case report was that by Vernon Mason [4] from this clinic in 1922. Emmel,[5] in 1917, carried out experimental studies on the blood from Cook and Myer's case and made the following observations: "About one-third of the erythrocytes were greatly elongated. Nucleated erythrocytes and punctate basophilia were always present. Phagocytosis of erythrocytes was found in the peripheral circulation. In culture preparations a great number of the disk-shaped corpuscles became elongated and crescentic and the tips of the crescentic forms frequently extended to tapering hairs. At one period of the study the crescentic forms disappeared from the circulation, but in the culture preparations the erythrocytes still manifested the tendency to assume elongated and crescentic forms. Similar preparations from normal blood and from cases of pernicious anaemia, chlorosis and myelogenous leukaemia gave only negative results. In culture preparations made by placing erythrocytes from a case

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of pernicious anaemia in the patient's serum, some of the red corpuscles assumed characteristics comparable to those observed in like preparations of the patient's blood." Finally, they conclude that "the culture reaction of the erythrocytes appears to be specific for this case of anæmia, and that the manner in which the red blood corpuscles are transformed into the sickle-shaped elements suggests that the phenomenon is in part at least due to an accentuated or abnormal activity of the same factors which in normal hematogenesis are involved in the transformation of the original spherical erythrocyte into a biconcave disk-shaped form."

Three cases were detected in this clinic between May 1922 and November 1922. This disease is evidently not very rare, and it seems probable that it is overlooked or mistaken for some other disease.

The object of this paper is to report 14 new cases, two of which will be described in detail. Six other cases have been diagnosed but have not been reported here on account of incomplete records.

CASE REPORTS

Case I. *C. T.*, colored, female, age 14.—Admitted Nov. 7, 1922. Has had one previous admission, March 23, 1917.

Admitted March 23, 1917, with the complaint of "rheumatism."

Family History.—Mother, sixty, has "heart trouble." Father, seventy, has "rheumatism" and "heart trouble." Three brothers, ages 44, 38 and 20 years, and three sisters, 45, 32 and 22 years, living. Brother, 20 years old, has rheumatism and heart trouble. One sister and one brother died in infancy—cause unknown.

Personal History.—The patient cannot remember having been strong and robust. She has always felt generally weak and has become worse during the past year. Before the age of 10 years she had "yellow jaundice" and says her eyes have been a little yellow ever since. At 10 years of age she had diphtheria but without complications. Pneumonia two years ago (1915). Frontal and parietal headaches frequently since childhood, during which attacks there is double vision; occasional attacks of giddiness and fainting; and "sore throat" frequently. Night sweats for last year. Appetite poor. Jaundice three years ago, duration not known. For the past year monthly attacks of abdominal pain one hour after eating and lasting for about one hour. Vomiting sometimes follows. Has not begun to menstruate.

Present Illness.—Onset five years ago with headaches and swelling of ankles with pain and tenderness. In two weeks the inflammation in the ankles began to subside and swelling and pain appeared in both knees. She remained in bed for one month and improved; but later she had another mild attack, which did not confine her to bed. She has had numerous mild attacks since. Last summer pain developed in her left "hip" and then in her right, but there was no œdema; she became short of breath and palpitation was noticed. Two months before admission there was precordial pain and swelling of the legs and abdomen, which disappeared in three days. On admission the patient was weak and tired very easily on exertion. Nycturia (3) during last month. Enters hospital for stiffness of right hip.

Physical Examination.—Temperature 100.4° F. Pulse 140, respiration 22, blood pressure 104/68.

Rather poorly developed for her age; height 5½ feet, weight 120 lbs. Tonsils large, ragged and pitted. Thorax infantile in type. Lungs clear. Heart enlarged to left; loud ringing systolic murmur at apex well transmitted. Abdomen shows nothing abnormal. No œdema. Reflexes normal. Slight limitation of motion in right hip-joint. Tenderness over head of femur and great trochanter.

Laboratory Examinations.—Blood findings shown in Chart III. Wassermann reaction negative. Urine 1012, acid, albumin 2 plus. Microscopical examination negative. X-ray of hip; some destruction of the head and acetabulum with a fracture of the epiphysis, indicating an infection and probably an injury.

Diagnosis on this admission.—Arthritis of hip. Mitral insufficiency, infantilism.

Second admission.—November 7, 1922. Age 19. Complaint.—Sore on foot (3 months' duration).

Since her discharge from hospital the patient has had colds every fall up to last year when her tonsils were treated. Occasional night sweats and some oedema of ankles during the last five years. Appetite never good. Always uses care in eating, as heavy foods often cause abdominal pains and sometimes nausea and vomiting. All gastric distress relieved by vomiting. Bowels regular. Nycturia (1) for the past five years. Menstruation began at the age of seventeen. There is no regularity of appearance, the intervals vary from two weeks to two months. Onset of menstruation is accompanied by weakness, dizziness and severe hypogastric pain which abate somewhat but do not disappear when the scant flow begins. The flow usually lasts two days, during which time patient is confined to bed.

Present Illness.—Three months ago the patient noticed on the dorsum of the left foot a vesicle which might have been disregarded except for unusual tenderness. By the following morning the vesicular contents had escaped. Two days later a tender throbbing swelling with increased temperature appeared at the site of the vesicle. Ulceration with profuse discharge appeared four days later. The diameter of the ulcer was about 1.5 cm. and did not increase. The constant localized burning pain with itching was intensified at night and sufficiently severe to prevent sleep. The patient states there has been an exaggeration of general symptoms and night-sweats for two weeks. There has been some photophobia and inability to read except for short periods. She states that the "whites of her eyes are always a little yellow." The appetite has been poor since August, 1922. Anything but light food may cause severe abdominal cramps, sometimes nausea and vomiting. Nycturia (2). Menstrual periods are more irregular, and dysmenorrhoea has increased. There has been a marked general weakness.

Physical Examination.—A moderately undernourished colored girl, not acutely ill. Over the lateral aspect of the dorsum of the left foot is a tender punched-out crater-like ulcer 2 cm. in diameter with a distinct margin (Fig. 5). The base is covered with ragged, grayish yellow exudate surrounded by an area of pigmentation and some œdema. There is a slight general glandular enlargement. The scleræ have a greenish yellow tint. Dental caries and moderate pyorrhoea. The m.m. are pale. Eyegrounds normal except for pallor. Lungs clear; heart slightly enlarged to the left. At the apex there is a short, soft blowing systolic murmur almost replacing S_1, poorly transmitted to axilla, and heard over precordium and at base. P_2 greater than A_2 and is accentuated and split. Pulse 100. Blood pressure 102/65.

The abdomen reveals nothing abnormal. The extremities are normal except for ulcer. Reflexes active and equal. External genitalia infantile, pubic hair scant; vagina admits two fingers, cervix small, uterus extremely small.

Course of disease.—The patient remained in the hospital for three weeks. The ulcer slowly healed after daily cleansings and applications of zinc oxide ointment. The pulse varied between 70 and 120 and the temperature between 96° F and 101° F. The

heart remained as described. The blood pressure remained between 108 systolic and 70 diastolic. The color of the scleræ remained without change.

Laboratory Examinations: Urine; low specific gravity; concentration test, specific gravity never exceeded 1012. Albumin, trace to a 2 plus; urobilin present; granular and cellular casts always present. Phthalein excretion 28% during first hour and 10% during second hour. Gastric analysis: HCl deficit of 23% and combined acids of 67%; other examinations negative. Vital capacity varied between 28% and 52% below normal. The Wassermann test was repeatedly negative. Von Pirquet reaction negative. Three blood cultures negative. Cultures from leg ulcer showed hæmolytic streptococcus, but no mycosis. No spirochætæ found in dark field preparations. The chemical analysis of the blood was as follows:—in mgm. per 100 c.c.; N.P.N. 18.6, blood creatinin 1.26, blood chloride 530, blood uric acid, 4.10, blood inorganic phosphorus 2.50; serum calcium 10.0, plasma CO_2 C.P. 48.8, vols. per cent. The serum protein as determined by refractometer 8.5%; as calculated from nitrogen content 8.5%. The red cells showed a slight decreased resistance to varying strengths of isotonic salt solutions. Vital stained cells varied from 5% to 30%. Histological examination of ulcer showed chronic inflammatory reaction. Blood microscopical examinations are shown in Chart III. Phagocytosis of the red blood cells was seen on November 17, 18 and 19 (Fig. 3). Van der Bergh test for bilirubin in the blood serum showed a high bilirubin content (1-45,000). Repeated stool examinations were negative for ova or parasites.

Case II.* *J. S.*, colored, male, age 17.—Six previous admissions to the medical service and two admissions to the surgical service of this hospital, with the same complaint—weakness and ulcers on legs.

Family History.—Father 55 years old, living and well. Mother died at fifty-two—cause unknown. One brother, 30 years, living and well. Two brothers dead, causes unknown. Three sisters, 18, 22, and 25, living and well. Three sisters dead. One died at the age of 25 years, of "convulsions." Cause of death of others unknown.

Personal History.—Health always poor. Diphtheria at 20 years. The patient's mother told him that he had rheumatism in early childhood. Has dull frontal headaches occasionally; susceptible to frequent sore throats. At 15 years of age had dizziness and fainting spells. Shortness of breath on exertion. Night sweats during period of cold; occasional palpitation. Appetite good with occasional indigestion. Nycturia (1). No history of Neisser or syphilis. Has always complained of weakness and occasional pains down back of legs.

Present Illness.—Began in 1910 when patient had several sores on his right ankle which became worse and appeared on the other ankle. The sores were oval in shape and were about the size of a quarter. Two sores were present on the left leg near the ankle and three on the right leg in the same area. Treatment at home was futile, and the patient was admitted to this hospital on January 25, 1911, complaining of leg ulcers and epistaxis. Just previous to admission epistaxis, weakness, and shortness of breath increased. The patient remained in the hospital until February 24, 1911; at this time the ulcers had healed and the other symptoms had disappeared.

While working, after his discharge from the hospital, he struck his left foot against the sharp edge of a box, after which sores reappeared on both ankles. He continued to work, but six months later returned to the Johns Hopkins Hospital Dispensary for treatment. The ulcers did not heal readily and the patient was re-admitted to the hospital on February 8, 1913. During this stay in the hospital he received several doses of salvarsan (0.4 gm.) without any effect on ulcers. Ulcers healed under simple palliative methods and the patient was discharged on February 28, 1913. He went to work again but contracted diphtheria and was readmitted to the hospital on April 11, 1913. He received one blood transfusion and was discharged improved on May 22, 1913. He was unable to work on account of pains in the elbows, shoulders and knees, each joint being affected at a different time. These never became red or swollen. The former symptoms —weakness, dyspnoea on exertion, and ulcers—reappeared. He was readmitted to the hospital on September 12, 1914. Following rest in bed and local application of ointment to the ulcers all symptoms again disappeared and the patient was discharged on November 4, 1914. After three months ulcers reappeared on the ankles. The patient became very weak and unable to work, his feet became swollen after walking and there was a marked polydipsia. He was readmitted to the hospital on March 19, 1915 and remained for 3 months. One transfusion was given. All symptoms disappeared and he was discharged on June 15, 1915. He attempted work but the ulcers on the legs reappeared and he was again admitted to the hospital on December 4, 1915. The treatment this time was the same as during his previous admission and the ulcers healed. He was discharged on January 28, 1916.

Physical Examination.—An undernourished, pale, colored man, with slight general glandular enlargement, greenish yellow scleræ, dental caries and marked pyorrhœa. Eye-grounds normal except for pallor. Lungs clear except for slight dullness at right apex but no râles are heard. Shocks of both heart sounds felt over body of heart and at apex. P.M.I. diffuse and wave-like, occupying V and VI i.s. and extending 13 cm. to left in VI i.s. R.C.D. 13.5 cm. in VI i.s. by 3 cm. in IV i.s. A blowing systolic murmur heard at apex, over the body of heart and at base. The murmur is transmitted faintly to the axilla. There is a proto-diastolic gallop rhythm. P_2 slightly accentuated and split, A_2 bell-like in quality. A systolic murmur is heard in vessels of neck. Pulse 96. Blood pressure 90/55. The liver edge is felt 3fb below C.M. in nipple line. Spleen not palpable. Pubic hair scant and testicles small. Reflexes are active and equal. Three centimeters above right ankle are two round "punched out" ulcers. White scars on both legs. The skin is glistening and darkly pigmented around both ankles extending a short distance up the legs. Slight œdema of ankles.

Laboratory Examination.—(Taken from all admissions.)

The urine, low specific gravity never greater than 1012. Albumin, a trace to a 3 plus; urobilin and granular casts always present. Phthalein test on three different admissions averaged 15% of the dye excreted in first hour and 16% in second hour. Gastric analysis on third admission; free HCl 37%; total acidity 53%. On last admission; HCl deficit 10% and combined acids 40%. Other examinations negative. Stool examinations revealed no ova or parasites. Wassermann tests repeatedly negative. Opthalmic tuberculin test negative. Repeated blood cultures negative. Cultures from leg ulcers revealed nothing definite. The red cells showed a slightly decreased resistance to varying strengths of isotonic salt solutions. Vital stained cells varied between 10% and 35%. Fresh preparations of blood showed that 99% of the erythrocytes acquired bizarre forms in 24 hours. Other blood findings are shown in Chart IV.

Course of Disease.—The patient developed tuberculosis during the summer of 1916; previously there had been no indication of this disease. He was sent to the City Hospital at Bay View, Baltimore, Maryland, where he remained until death on March 28, 1917, at the age of twenty-four years. The autopsy record and specimens were lost except as described on page 340.

* This case is reported through the kindness of Doctor Sydney R. Miller.

Case III. *J. T.* colored, male, aged 70. Married, seven children. No history of any serious illness. Occasional attacks of "rheumatism." Has worked hard. Only by examination of fresh preparations of his blood was the disease detected. In these preparations, 25% of the erythrocytes acquired bizarre forms after standing 24 hours. Other blood findings were normal. (Chart V.)

Case IV. *S. N.*, Eldest child of J. T., case 3. Colored female, aged 45. Married, seven children. Always in good health. 25% of her erythrocytes acquired bizarre forms in 24 hours. Other blood findings normal. (Chart V.)

Case V. *E. T.*, second child of J. T., case 3. Colored female, aged 44. Married, four children. The patient has always been fairly healthy with occasional periods of weakness. Easily fatigued by hard work. Occasional attacks of "rheumatism." 50% of erythrocytes acquired bizarre forms in 24 hours. Other blood findings were normal except for a mild grade of secondary anemia. (Chart V.)

Case VI. *W. T.*, Third child of J. T., case 3.—Colored male, aged 38. Married, five children. At times he "had to stop work for a week or two and rest, because of weakness;" at other times does fairly hard work. Occasional attacks of "rheumatism." 50% of the erythrocytes acquired bizarre forms in 24 hours. Only other unusual findings in the blood showed a mild grade of secondary anaemia. (Chart V.)

Case VII. *N. T.*, sixth child of J. T., case 3.—Colored male, aged 20. Unmarried. Has never been able to work hard or attend school regularly. Three years ago an ulcer appeared on his right leg but disappeared after treatment by a physician. He is always weak and tired, and is able to do only the lightest kind of work. Fresh preparations of his blood show that 99% of the erythrocytes acquire bizarre forms in 24 hours. Hb. 40%. R.B.C. 2,700,000. W.B.C. 15,000. Differential formula shows a slight increase in polymorphonuclear neutrophile cells (75%). Platelets, 350,000. Phagocytes found in the peripheral circulation. (Chart V.)

Case VIII. *E. N.*, second child of S. N., case 4.—Colored female, aged 14. Never had any severe illness. Attends school regularly. 25% of the erythrocytes acquire bizarre forms in 24 hours. Other blood findings reveal nothing unusual. (Chart V.)

Case IX. *J. N.*, third child of S. N., case 4.—Colored male, aged 12. Has never been strong. Attends school irregularly, owing to periods of ill health consisting principally of weakness and fatigue. 50% of erythrocytes acquire bizarre forms in 24 hours. Hb. 60%. R.B.C. 3,500.000. W.B.C. 10,000. Differential formula shows a slight increase in small lymphocytes (32%). No other unusual findings in the blood. (Chart V.)

Case X. *R. N.*, sixth child of S. N., case 4.—Colored female, aged 5. Never any symptoms. Plays like other children of the same age. 25% of erythrocytes acquire bizarre forms in 24 hours. Other blood findings normal, for this age. (Chart V.)

Case XI. *E. T.*, second child of E. T., case 5.—Colored male, aged 10. Has never been well. "Lays around all the time." 99% of the erythrocytes acquire bizarre forms in 24 hours. Hb. 35%. R. B. C. 2,800,000. W.B.C. 12,000. Differential formula reveals nothing unusual. Other blood examinations not performed. (Chart V.)

Case XII. *G. T.*, third child of W. T., case 6.—Colored female, aged 9. Usual diseases of childhood, otherwise always healthy. Attends school regularly. 25% of the erythrocytes acquire bizarre forms in 24 hours. Other blood examinations normal. (Chart V.)

Case XIII. *A. T.*, fifth child of W. T., case 6.—Colored male, aged 3. Apparently normal healthy child. 25% of erythrocytes acquire bizarre forms in 24 hours. Other blood examinations normal. (Chart V.)

Case XIV. *G. C.*, No relation to other cases. Colored female, aged 28. Married, four children. Has had fairly good health but has never been able to work hard. 50% of the erythrocytes acquire bizarre forms in 24 hours. Hb. 65%. R.B.C. 4,000,000. W.B.C. 9,000. Differential formula shows nothing unusual. (Chart V.)

No intestinal or blood parasites found in any of the cases.

Experimental Studies

Certain experiments to be described were carried out with the blood from patients having various types of the disease.

Behavior of the erythrocytes in fresh preparations. (1) Fresh preparations of the blood from Patients I, II, VII, and XI, each with the severe form of the disease, were made by bringing a clean coverslip in contact with a drop of blood from the finger-tip, mounting immediately on a glass slide and rimming with petroleum jelly. Immediate microscopical examination revealed little change in the red blood cells, although an occasional sickle-shaped cell was seen. After standing for six hours the preparations revealed practically three quarters of the cells to have acquired the sickle or bizarre form (Fig. 4). At the end of twenty-four hours practically all of the cells had changed to a sickle or bizarre form with long thread-like processes extending from the cells (Fig. 4). After three days to six weeks the cells assumed the spherical form (Fig. 4). In preparations from patients with mild symptoms, 75% of the cells assumed a sickle or bizarre form, and in those from patients without symptoms 25% of the cells changed to sickle or bizarre forms.

(2) The blood of the patients who were without symptoms, and of those who showed mild, and severe symptoms of the disease, was placed in (a) a solution of 1.25% citrate in M/8 NaCL (isotonic salt solution), (b) in physiological salt solution; and (c) a third series was set up in which the whole blood was oxalated. From these solutions fresh preparations were made. The red cells behaved in exactly the same manner in the three series as with the patient's whole blood in experiment 1.

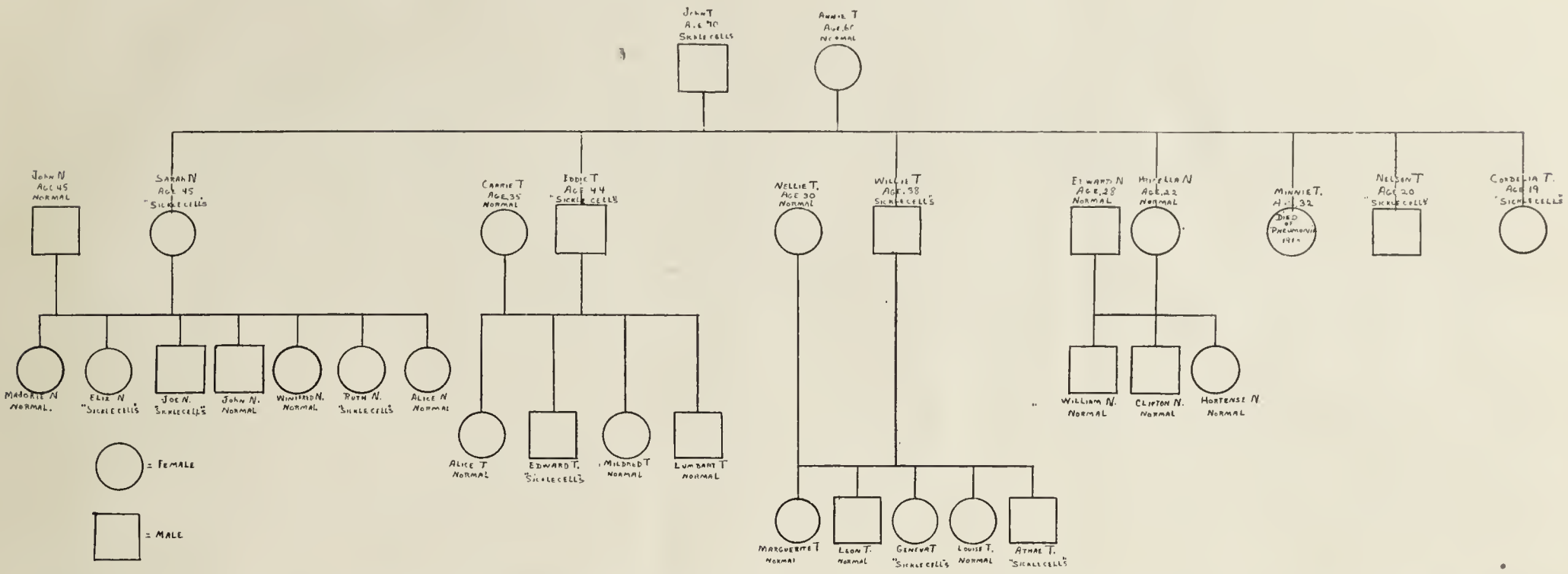
(3) Red blood cells from each form of the disease were first washed in isotonic salt solution and then placed in normal blood serum from a member of the same blood group. Fresh preparations were made and sealed. The sickling and bizarre forms appeared in the cells of each type of the disease in the percentage described in experiment 1.

(4) Normal red blood cells of the same blood group as the patients were washed three times in isotonic salt solution, and suspended in the patients' sera of the three types. Fresh preparations were made and sealed. The normal cells in these preparations remained unchanged during a week's observation.

(5) A drop of fresh blood from cases of pernicious anaemia, secondary anaemia, myeloid and lymphoid

CHART I.

Occurrence of "Sickle Cells" in the family of Case I.



leukæmia, congenital hæmolytic jaundice and Banti's disease were set up in fresh preparations as described in (1), but no change was observed in their cells after one week.

From these experiments it may be concluded that the sickling is an inherent property of the red blood cells and that the patients' sera are without effect on normal erythrocytes.

Effect of light on the erythrocytes.—Exp. 6. Fresh preparations were made from patients of the three types, and, as soon as sealed, were placed in black, light-proof boxes in a dark cupboard. Similar preparations made at the same time were kept in the light. The sickling and bizarre forms took place just as rapidly in the dark, but the reversal to spherical form was accomplished more quickly. All the cells in the preparation kept in the dark had reversed within two days, whereas the controls remained sickled for a period of three days to one month.

Exp. 7. The bloods of one hundred negroes were obtained and fresh preparations were made, but no changes were apparent in the red blood cells in the course of one week.

Exp. 8. One cubic centimeter of a 50% suspension of washed red blood cells from a patient with severe symptoms was injected intravenously into each of four rabbits. No abnormal changes took place in these animals.

Discussion

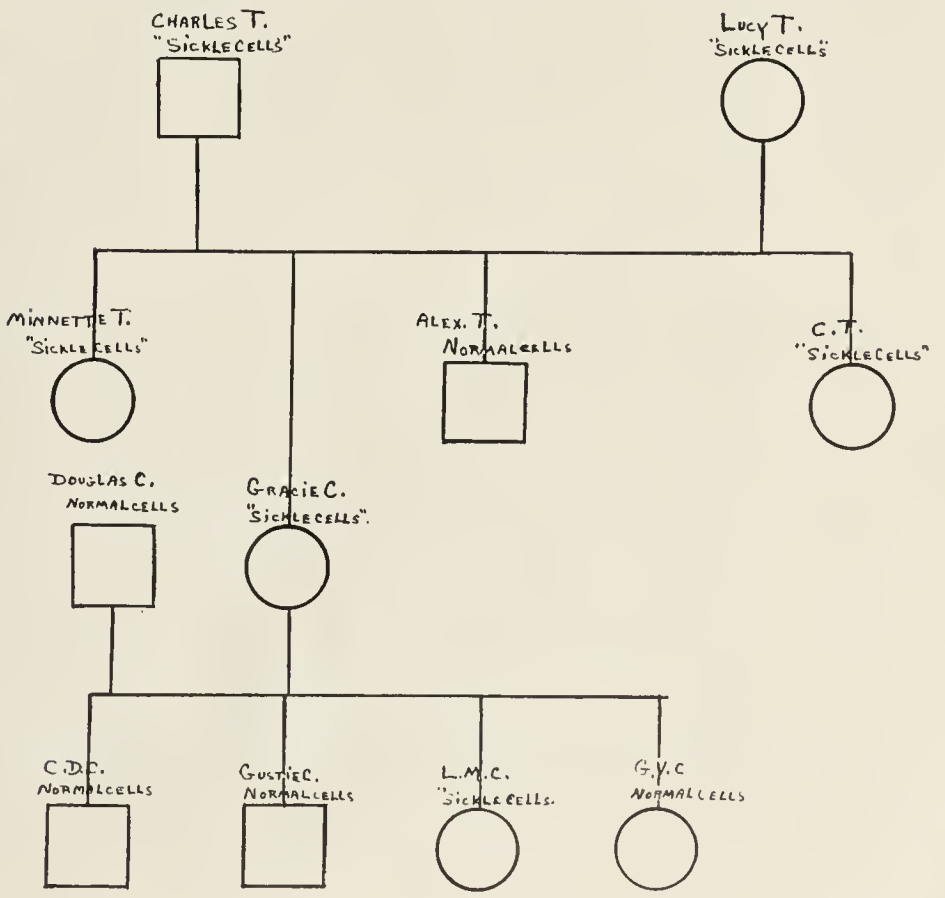
Sickle cell anaemia may be defined as a hereditary disease, transmitted by and occurring in both males and females with equal frequency, in which the red cells of the blood acquire a characteristic sickle or crescent shape *in vitro*. The disease is characterized by certain typical clinical symptoms, especially those of anaemia and leg ulcer. All cases so far observed have occurred in the negro race. The localities where this disease has been found are as follows:—4 cases from Virginia, 1 from Missouri, 1 from the West Indies, and 2 cases from Maryland. The ages of the patients were between 3 and 25 years.

Etiology.—At first this disease was thought to be a manifestation either of syphilis or of tuberculosis, but from observations made upon three generations of a family, it is plainly shown that the disease is familial (Chart I).

In this genealogical tree the male transmitted the disease in the first generation, but in the second generation the disease was transmitted both by males and females, and in another genealogical tree by both mem-

CHART II.

Occurrence of "Sickle Cells" in the family of C. T.



bers of the first generation.* (Chart II.) Apparently the "sickle cell" condition in man is inherited according to the Mendelian law for the inheritance of a single factor. One interesting feature in this inheritance is the fact that the sickle cell condition is dominant over the normal condition. An analysis of these genealogical trees is to appear shortly in collaboration with Dr. W. H. Taliaferro of the School of Hygiene, Johns Hopkins University.

Pathology.—No post-mortem findings have been reported.†

The leg ulcers in these cases have the round, punched out and indurated appearance of syphilitic ulcers, for which they are very often mistaken. Histologically, the papillæ of the epithelium near the edge of the ulcer extend downward to an abnormal degree, forming a rather course network. In the corium the connective tissue is increased above the normal amount. The walls of the rather numerous blood vessels are not abnormal. There is a diffuse scattering of mononuclear wandering cells throughout the tissues. These cells are of the ordinary variety characteristic of chronic inflammation, but in most places the plasma cell and large mononuclear wandering cell types predominate over the small lymphoid type. Scattered among these mononuclear cells are found a few neutrophilic polymorphonuclear leucocytes. In places this wandering cell infiltration is concentrated about small blood vessels and atrophic sweat glands. Many other blood vessels are entirely free from perivascular infiltration. On the edge of the ulcers the epithelium ceases abruptly without any tendency to downward growth. The base of the ulcer is composed of granulation tissue possessing no characteristics suggestive of any specific etiology. The surface is clean, there is a total absence of a necrotic membrane overlying it. A few sickle-shaped red cells may be seen in the areas where there are red blood cells (Fig. 1).

Blood findings.—The blood flows and coagulates normally in all forms of the disease. The erythrocytes vary from 1,500,000 to 4,000,000, and the hæmoglobin is also reduced, varying between 30% and 63%. The color index is usually normal or high. The white cells of the blood are slightly increased, varying between 10,000 and 20,000, except in the presence of some intercurrent infection, in which event counts as high as 35,000 have been observed. Stained films show a moderate anisocytosis and poikilocytosis which occurs more frequently during relapses (Fig. 2). The poikilocytosis consists mostly of crescent or sickle-shaped cells with an occa-

*Guthrie, C. G. and Huck, J. G.:—"On the Existence of More Than Four Isoagglutinin Groups in Human Blood." *Johns Hopkins Hospital Bulletin*, February, 1923.

† Case II reported in this paper was complicated by tuberculosis from which the patient died, and at section revealed a hyperplasia of the bone-marrow with an atrophy of the spleen. The spleen measured 2½ inches in length. There was a general glandular hyperplasia with a tuberculous pleurisy and pulmonary tuberculosis. The heart was slightly enlarged. The kidneys were smaller than normal; the surface was irregular and the capsule was adherent to the kidney substance. On cross-section the cortex was thin and cortical radiations were not distinct. Records of other autopsy findings in this case were lost.

CHART III.

Blood Chart—Case I.

Date	3/23/17	3/29/17	11/7/22	11/8/22	11/10/22	11/13/22	11/16/22	11/21/22	11/24/22
Hb.	47%	45%	56%	57%	60%	53%	64%	65%	66%
R. B. C.	2,088,000	3,800,000	2,572,000	2,328,000	3,000,000	2,680,000	3,392,000	2,864,000	2,472,000
C. I.	1.1	.59	1.1	1.2	1.0	1.0	.96	1.1	1.3
Anisocytosis	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Poikilocytosis	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Basophilia	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate
Nucleated R. B. C.	2 Normoblasts	None	2 Normoblasts	1 Normoblast	4 Normoblasts	4 Normoblasts	6 Normoblasts 1 Intermediate	2 Normoblasts	None
W. B. C.	5,520	13,000	13,260	14,720	12,000	10,280	18,800	14,400	12,320
P. M. N.	76.6%	70.0%	74.7%	70.0%	69.3%	72.6%	65.6%	74.2%	61.8%
P. M. E.	1.5%	None	2.6%	5.0%	3.33%	5.7%	2.3%	1.4%	1.9%
P. M. B.	2.0%	3.0%	None	None	None	None	None	None	None
L. Lym.	2.0%	7.5%	2.6%	7.0%	None	4.0%	1.7%	0.1%	0.9%
S. Lym.	16.0%	14.0%	12.8%	11.0%	18.3%	33.0%	16.4%	7.4%	26.3%
L. Mono.	None	None	5.0%	3.0%	2.0%	2.0%	7.0%	7.0%	5.5%
Trans.	2.0%	5.5%	2.3%	3.0%	6.0%	3.0%	5.0%	8.4%	2.0%
Myeloblasts	None	None	None	None	None	None	None	None	None
Myelocytes, N.	None	None	None	1.0%	0.66%	1.7%	None	1.4%	None
Myelocytes, E.	None	None	None	None	0.33%	None	2.0%	None	1.6%
Myelocytes, B.	None	None	None	None	None	None	None	None	None
Path. Lym.	None	None	None	None	None	None	None	None	None
Platelets	Moderately Increased	Moderately Increased	Markedly Increased	490,000	470,000	480,000	472,000	Markedly Increased	450,000

CHART IV.
Blood Chart—Case II.

Date	11/2/10	12/22/10	1/9/11	1/12/13	2/1/13	4/6/13	4/12/13	4/18/13	4/30/13	1/20/14	9/12/14	9/17/14
Hb.	35%	42%	45%	30%	35%	35%	36%	35%	32%	30%	36%	35%
R. B. C.	2,840,000	1,552,000	2,020,000	2,080,000	1,800,000	2,800,000	1,912,000	2,076,000	1,920,000	1,888,000	1,812,000	1,760,000
C. I.	.62	1.4	1.1	.75	.94	.62	.94	.87	.84	.83	1.0	1.2
Anisocytosis	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Poikilocytosis	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Basophilia	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate
Nucleated R. B. C.	2 Normoblasts	None seen	None seen	4 Normoblasts	1 Normoblast	None seen	1 Normoblast	None seen	None seen	None seen	4 Normoblasts 2 Intermediates	2 Normoblasts
W. B. C.	25,000	27,000	20,000	15,000	11,000	8,000	11,440	30,000	33,280	14,040	19,450	14,900
P. M. N.	66.0%	83.0%	66.0%	66.8%	72.2%	83.0%	77.3%	73.3%	65.3%	72.6%	74.66%	74.66%
P. M. E.	1.5%	5.0%	None	2.8%	3.4%	None	1.0%	None	3.3%	1.8%	4.66%	2.66%
P. M. B.	None	None	None	None	0.6%	None	1.0%	0.33%	0.3%	None	1.33%	1.33%
L. Lym.	None	None	None	None	None	None	None	None	None	None	None	None
S. Lym.	15.0%	4.0%	22.0%	18.8%	20.8%	None	11.66%	17.6%	23.6%	16.5%	12.66%	15.33%
L. Mono.	2.5%	5.0%	8.0%	7.2%	1.4%	5.0%	7.33%	6.0%	5.3%	6.6%	4.66%	2.66%
Trans.	9.0%	None	4.0%	4.4%	0.6%	None	1.66%	2.6%	2.0%	2.1%	6.0%	3.33%
Myeloblasts	3.0%	None	None	None	None	3.0%	None	None	None	None	None	None
Myelocytes, N.	1.0%	3.0%	None	None	1.0%	1.0%	None	None	None	0.3%	None	None
Myelocytes, E.	None	None	None	None	None	None	None	None	None	None	None	None
Myelocytes, B.	None	None	None	None	None	None	None	None	None	None	None	None
Path. Lym.	None	None	None	None	None	None	None	None	None	None	None	None
Platelets	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased

CHART IV.—(Continued)

Date	9/29/14	10/6/14	11/9/14	3/22/15	3/30/15	4/6/15	4/13/15	4/18/15	4/19/15	4/20/15	4/27/15	5/4/15
Hb.	32%	31%	35%	36%	37%	38%	36%	44%	48%	48%	44%	40%
R. B. C.	1,650,000	2,062,500	2,080,000	1,812,000	1,384,000	1,412,000	1,680,000	2,368,000	2,384,000	2,464,000	2,208,000	2,096,000
C. I.	1.0	.77	.87	.87	1.4	1.3	1.1	.95	1.0	1.0	1.0	1.0
Anisocytosis	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Poikilocytosis	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Basophilia	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate
Nucleated R. B. C.	None seen	None seen	None seen	1 Normoblast	3 Normoblasts 3 Intermediates	2 Normoblasts	5 Normoblasts	4 Normoblasts	5 Normoblasts	6 Normoblasts	None seen	None seen
W. B. C.	10,809	12,800	17,000	12,400	11,440	11,440	12,700	9,240		14,960	9,560	13,560
P. M. N.	69.6%	72.0%	83.0%	48.0%	55.6%	64.0%	66.0%	62.5%	69.0%	84.4%	58.4%	59.5%
P. M. E.	1.6%	2.0%	None	0.6%	0.8%	1.6%	2.4%	None	1.5%	0.4%	3.2%	1.0%
P. M. B.	2.0%	3.0%	None	1.2%	1.2%	1.2%	None	None	None	0.8%	None	None
L. Lym.	None	None	None	None	None	None	None	None	None	None	None	None
S. Lym.	16.4%	21.0%	None	25.6%	23.2%	19.6%	20.4%	20.5%	16.5%	5.2%	30.8%	27.5%
L. Mono.	8.8%	2.0%	5.0%	19.3%	16.8%	10.0%	8.8%	13.5%	10.0%	6.8%	5.2%	8.0%
Trans.	1.6%	2.6%	None	4.6%	1.2%	3.6%	2.4%	3.5%	3.0%	2.4%	2.4%	4.0%
Myeloblasts	None	None	3.0%	None	None	None	None	None	None	None	None	None
Myelocytes, N.	None	None	1.0%	0.3%	None	None	None	None	None	None	None	None
Myelocytes, E.	None	None	None	None	None	None	None	None	None	None	None	None
Myelocytes, B.	None	None	None	None	None	None	None	None	None	None	None	None
Path. Lym.	None	None	None	None	None	None	None	None	None	None	None	None
Platelets	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased	Moderately Increased

CHART IV.—(Continued)

Date	5/12/15	6/4/15	6/9/15	6/15/15	12/4/15	12/9/15	12/22/15	1/7/16	1/24/16
Hb.	43%	38%	45%	35%	33%	34%	34%	35%	35%
R. B. C.	1,968,000	1,880,000	2,280,000	2,000,000	1,824,000	1,804,000	1,850,000	2,096,000	2,208,000
C. I.	1.1	1.0	1.0	.87	.91	.94	.94	.87	.79
Anisocytosis	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Poikilocytosis	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Basophilia	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate	Moderate Diffuse and Slight Punctate
Nucleated R. B. C.	1 Normoblast	None seen	None seen	None seen	6 Normoblasts 2 Intermediates	5 Normoblasts	12 Normoblasts	7 Normoblasts	2 Normoblasts
W. B. C.	10,720	14,600	11,500	12,750	14,120	11,960	12,500	10,760	10,760
P. M. N.	69.0%	65.0%	67.5%	74.0%	74.66%	75.25%	72.5%	75.0%	77.3%
P. M. E.	None	2.0%	1.5%	1.5%	0.44%	1.5%	1.0%	0.3%	0.3%
P. M. B.	1.5%	1.5%	1.5%	1.0%	0.2%	None	0.25%	0.3%	None
L. Lym.	None	None	None	None	None	None	None	None	None
S. Lym.	None	24.0%	22.5%	15.0%	17.77%	16.5%	19.25%	12.0%	12.6%
L. Mono.	None	5.0%	5.0%	7.5%	5.33%	4.75%	5.0%	3.0%	4.6%
Trans.	None	2.5%	2.0%	1.0%	1.55%	2.0%	2.0%	5.0%	4.6%
Myeloblasts	None	None	None	None	None	None	None	None	None
Myelocytes, N.	None	None	None	None	None	None	None	0.1%	None
Myelocytes, E.	None	None	None	None	None	None	None	None	None
Myelocytes, B.	None	None	None	None	None	None	None	None	None
Path. Lym.	None	None	None	None	None	None	None	None	None
Platelets	Moderately Increased	Markedly Increased	Markedly Increased	Markedly Increased	Markedly Increased	Markedly Increased	Markedly Increased	Markedly Increased	Markedly Increased

sional oat-shaped cell. There is a moderate grade of polychromatophilia consisting principally of the diffuse variety, although occasionally slight punctate basophilia occurs. Nucleated red cells of all varieties (normoblasts, intermediates and megaloblasts) may be found, but especially common are those of the normoblastic type. No Cabot rings or Howell-Jolly bodies have been found. The differential formula is not characteristic; the polymorphonuclear neutrophilic cells are normally increased, and at times one may find an increase of either the polymorphonuclear eosinophiles or the small lymphocytes. Myelocytes of the neutrophilic, eosinophilic or basophilic variety are found, but not constantly; these cells vary between 1% and 3% and occasionally form 7% of the total number of leucocytes. The neutrophilic myelocytes form the majority of abnormal white cells.

Cells of the large mononuclear variety are seen at times, phagocytizing red cells (Fig. 3). These are in all probability real phagocytes which wander into the blood-stream possibly from the spleen and liver, and do not belong to the large mononuclear—transitional group of cells. In fresh preparations one may observe these cells engulfing the erythrocytes. It is possible that this phagocytosis may have something to do with the anæmia. Sometimes phagocytes are seen in great numbers in the circulating blood, while at other times they are absent. Platelets are increased, varying between 300,000 and 500,000.

Fresh wet preparations of the blood, that have been sealed with petroleum jelly reveal a most interesting phenomenon. Immediately on examination of the preparation one finds only a few of the erythrocytes having a crescent or sickle shape, but after from 6 to 24 hours, from 25 to 100% of the cells, according to the severity of the disease, acquire bizarre forms (Fig. 4).

The resistance of the red blood cells to isotonic salt solutions shows a slight decrease below normal. The reticulated cells, as demonstrated by vital stains, are greatly increased and may reach 10% and 35%, indicating that the bone marrow is responding efficiently to the demands for increased blood formation. The bilirubin content of the serum is high.

The red blood cells, white blood cells, hæmoglobin, differential count, and platelets in the symptomless type of the disease may show nothing unusual, but one usually finds a moderate grade of secondary anæmia in the mild type.

Five combinations of agglutinin-agglutinogen content are represented in this series of 14 cases, as follows:—

Classification according to Guthrie & Huck[6]		Classification according to Blood Grouping of Moss.[7]
Agglutinin-agglutinogen combination	Number of cases	Group
O — ab	2	I
A — b	3	II
BC — a	4	III
ABC — o	4	IV
O — a	1	?

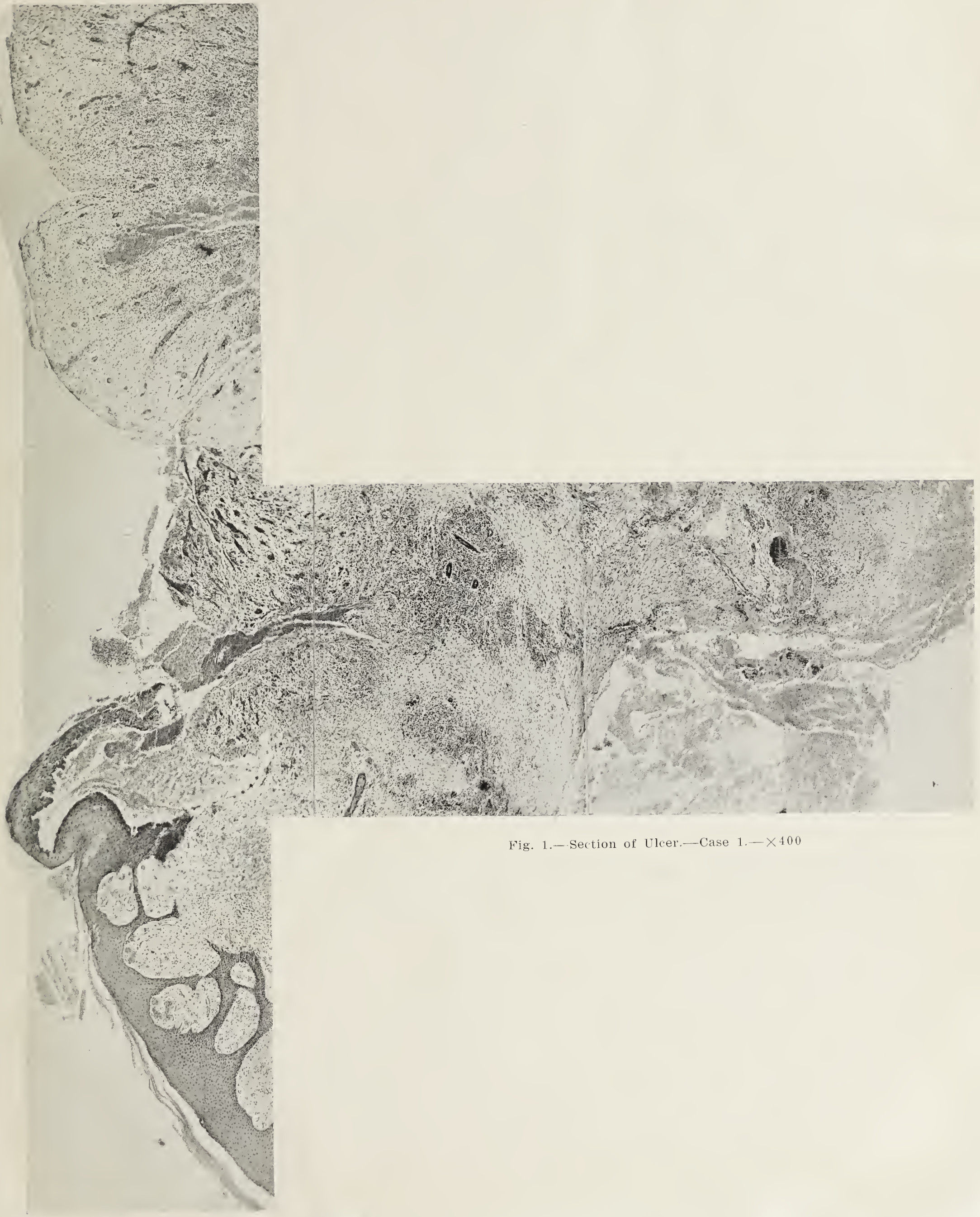


Fig. 1.—Section of Ulcer.—Case 1.—×400

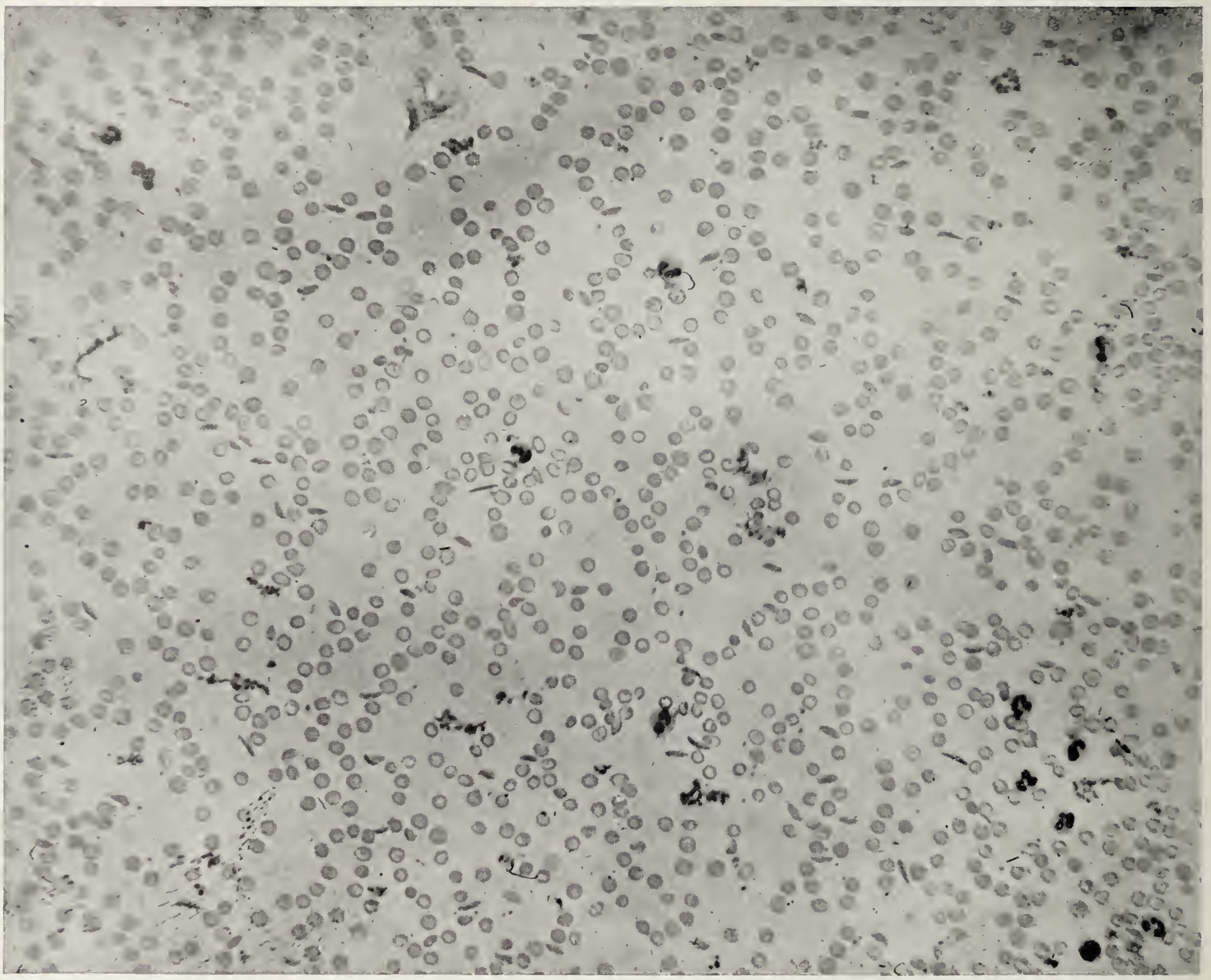


Fig. 2.—Stained Blood from patient with severe type.—×800

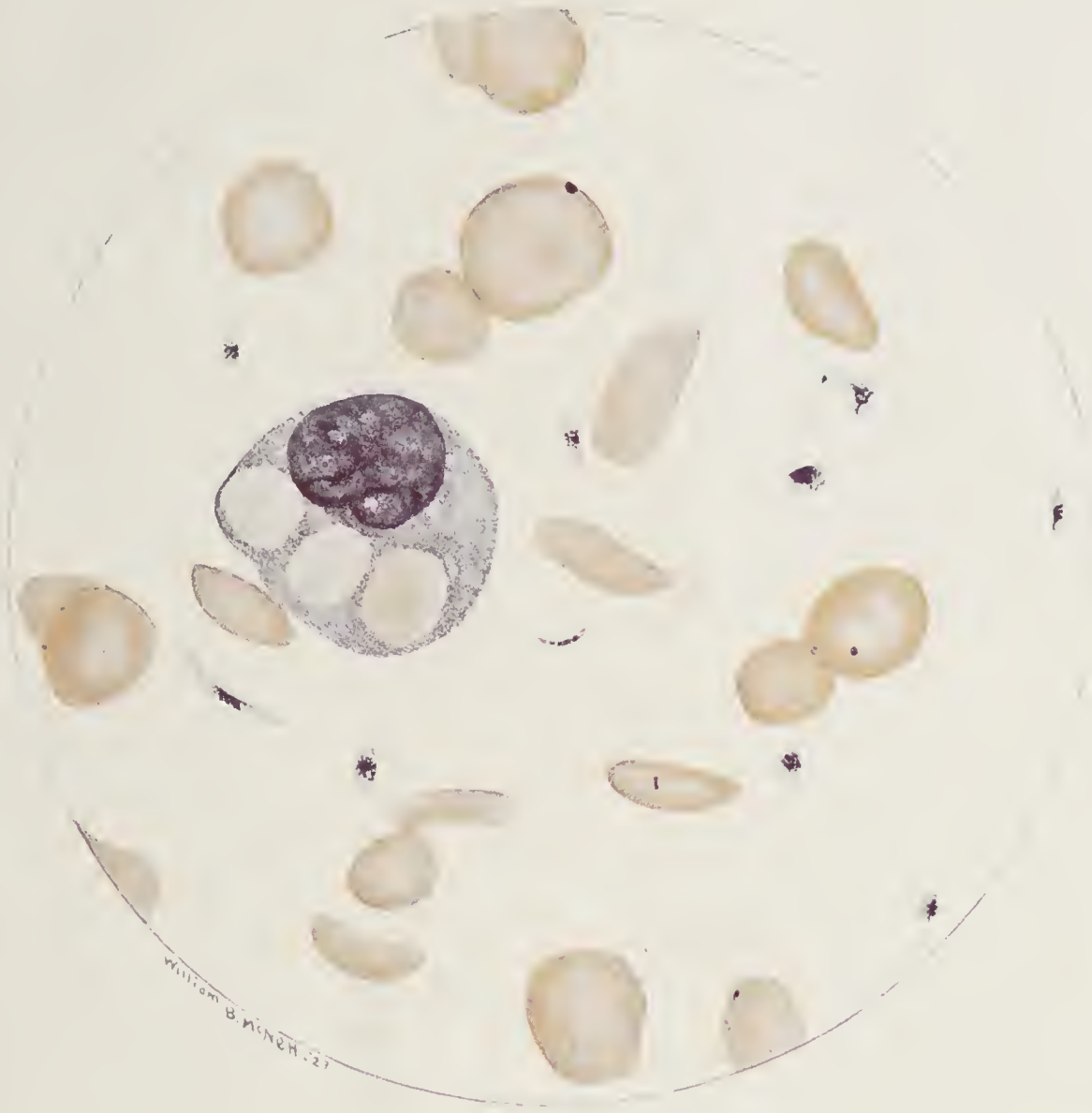
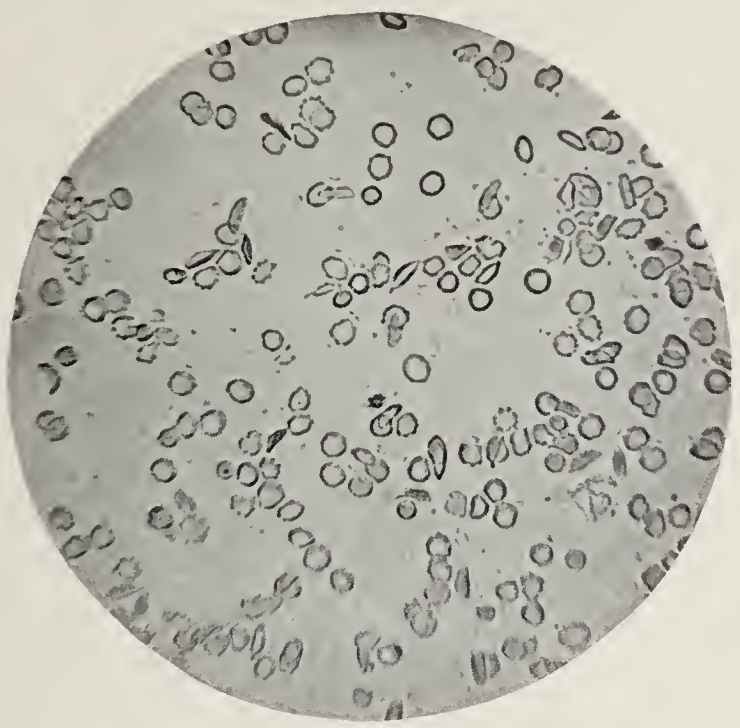
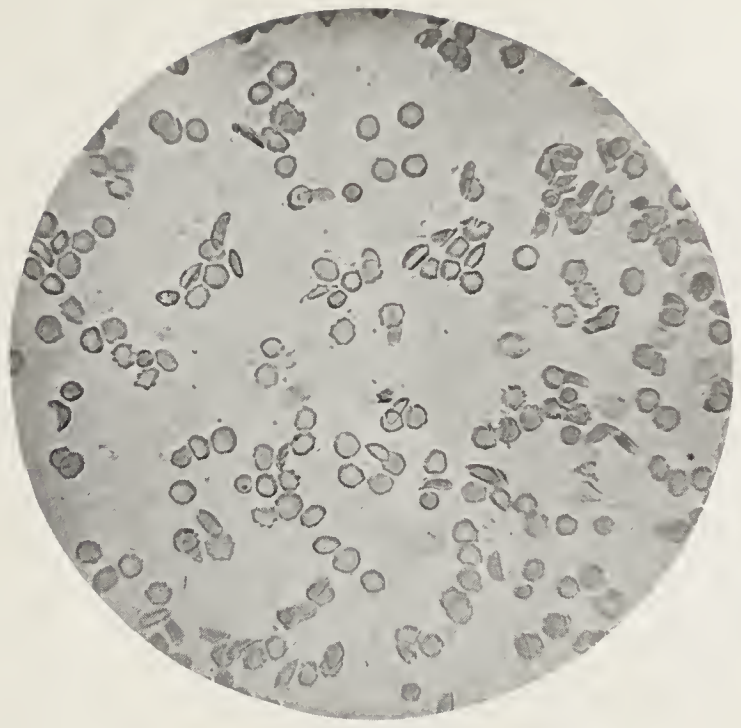


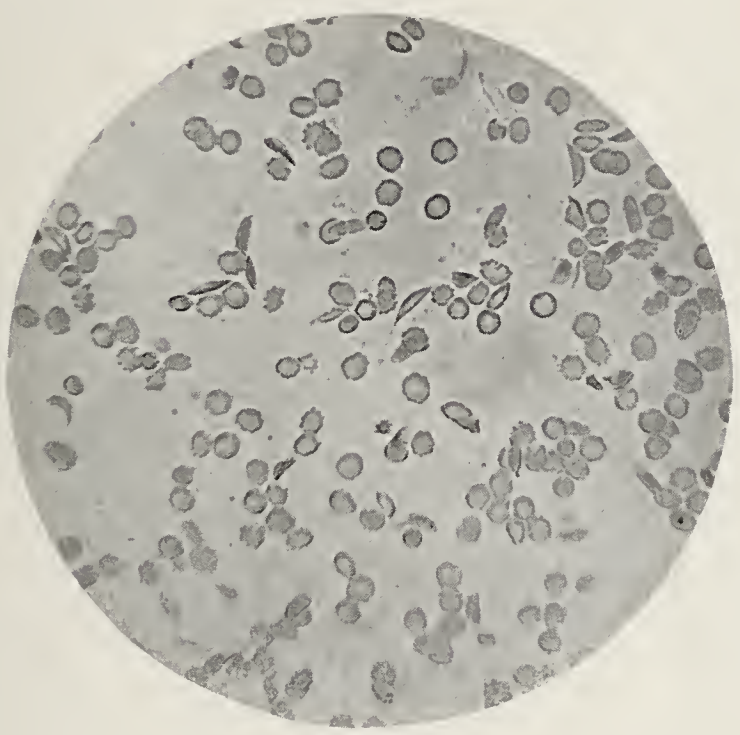
Fig. 3.—Phagocytes in circulating blood.



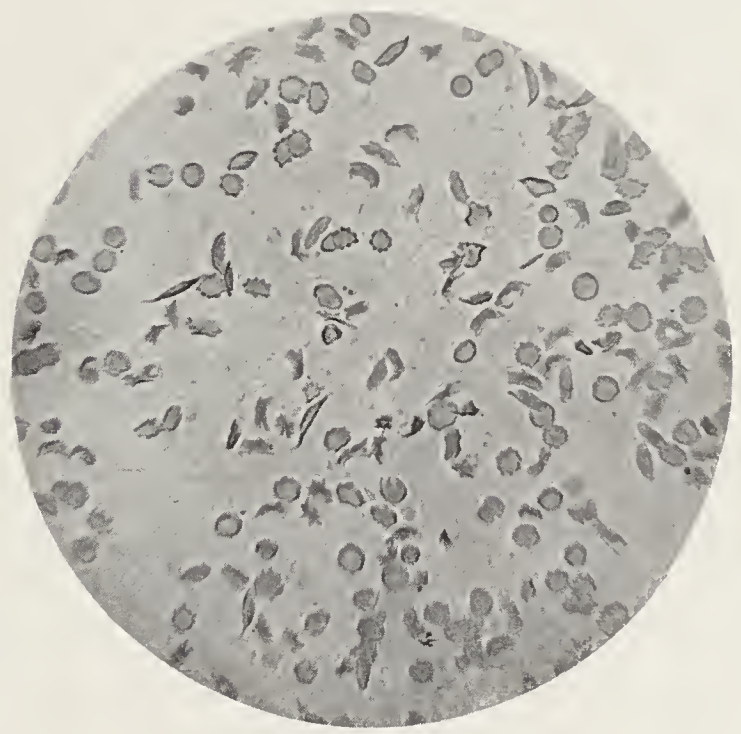
Immediately



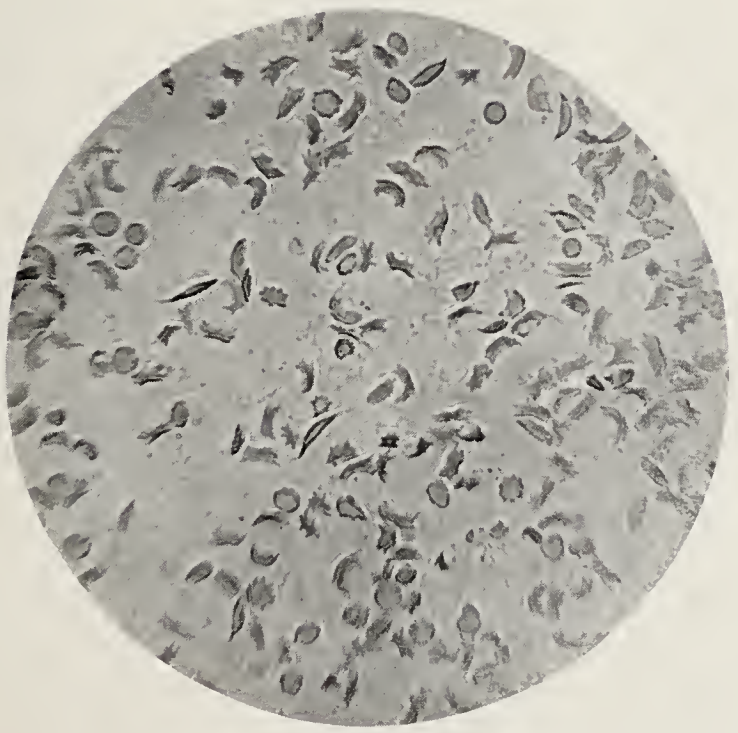
1 hour.



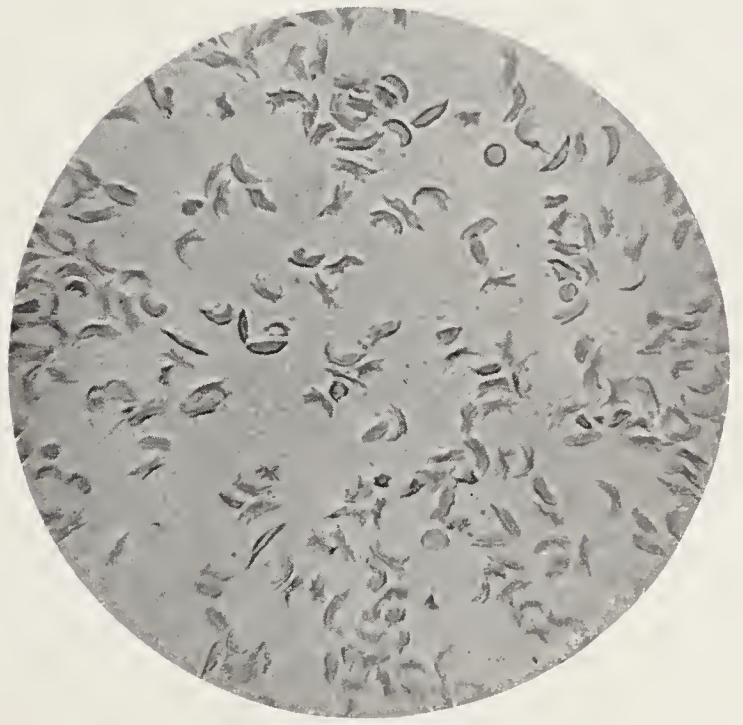
2 hours.



6 hours.

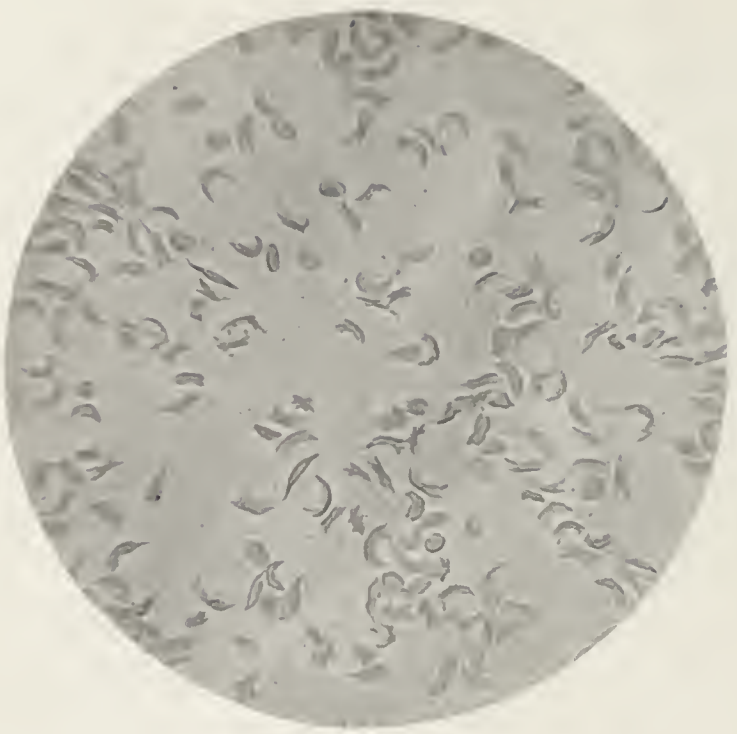


12 hours

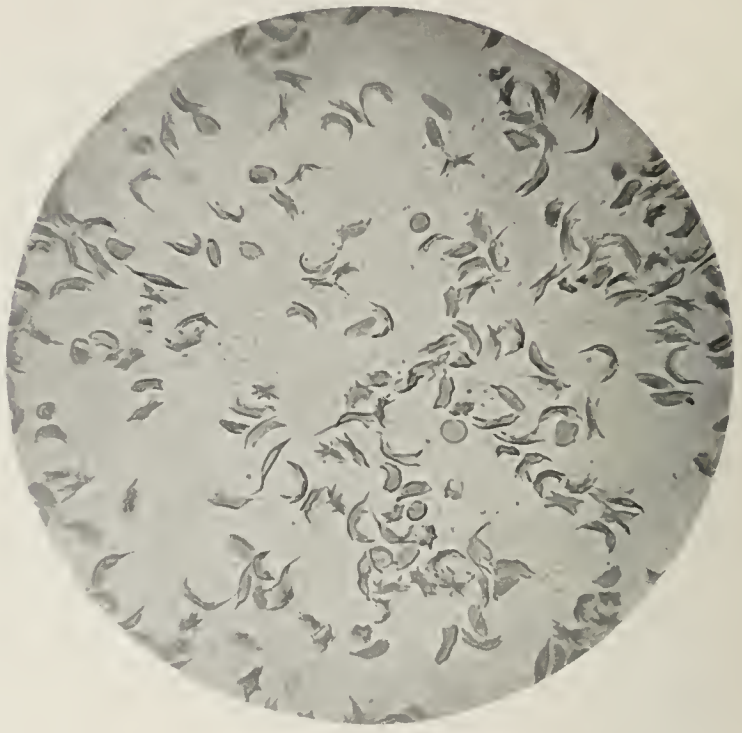


24 hours.

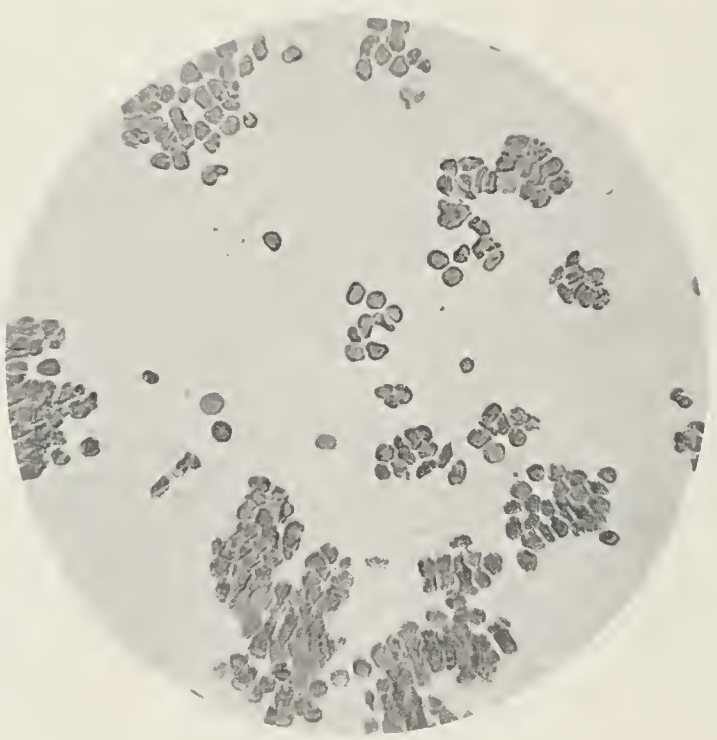
Fig. 4.—Photographs of fresh preparation showing changes at different hours.—×700.



36 hours.



48 hours.



72 hours.

Fig. 4.—Photographs of fresh preparation showing changes at different hours.—×700—(Continued)

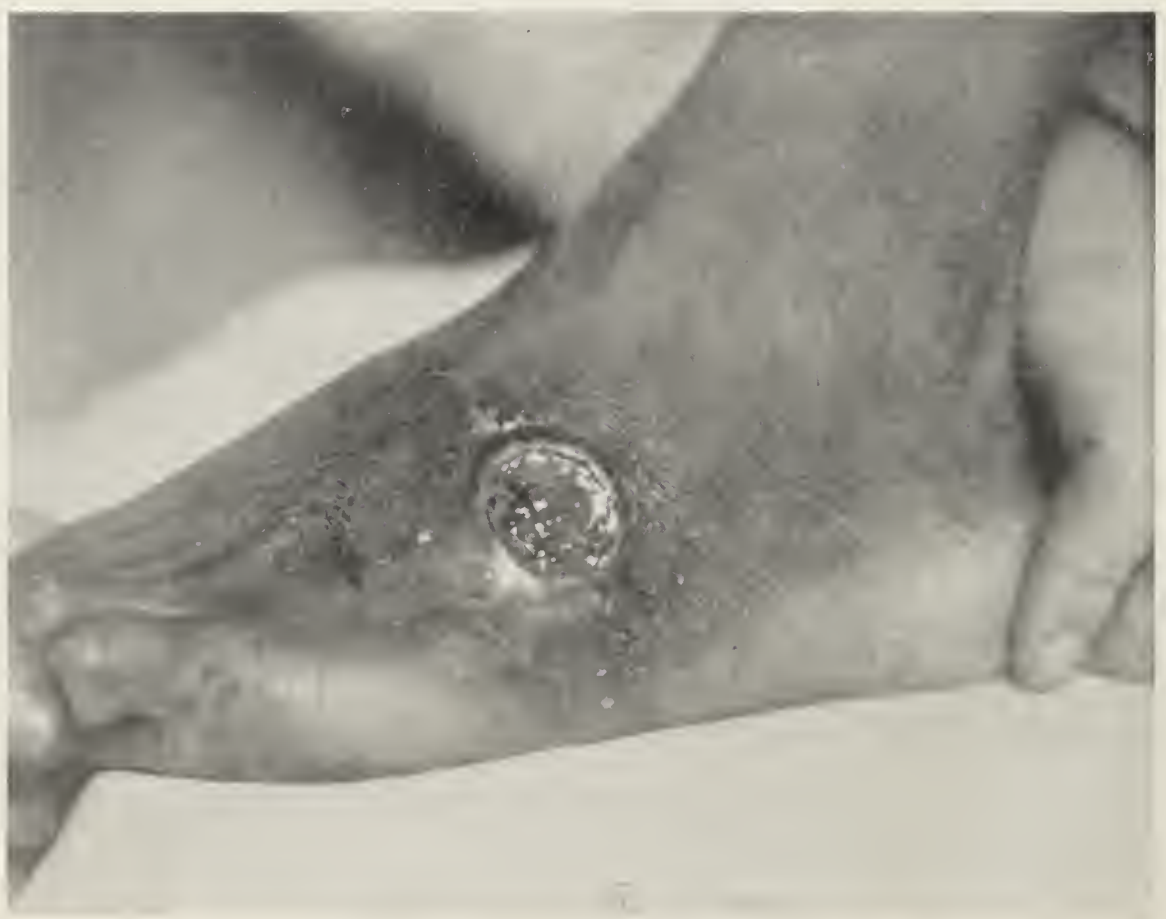


Fig. 5.—Ulcer—Case I.

CHART V.
Blood Chart—Cases III to XIV.

Type of Disease	Case No.	Hb.	R. B. C.	Color index	Anisocytosis	Poikilocytosis	Basophilia	Nucleated R. B. C.	W. B. C.	P. M. N.	P. M. E.	P. M. B.	L. Lym.	S. Lym.	L. Mono.	Trans.	Myeloblasts	Myelocytes N.	Myelocytes E.	Myelocytes B.	Path. Lym.	Platelets	% of Erythrocytes acquiring bizarre forms after standing 24 hrs.
Symptomless	III	90%	5,000,000	.9	None	None	None	None	8,000	65%	1%	0	1%	25%	3%	5%	0	0	0	0	0	258,000	25%
Symptomless	IV	82%	4,500,000	.9	None	None	None	None	7,000	67%	2%	1%	0	20%	2%	8%	0	0	0	0	0	275,000	25%
Symptomless	VIII	80%	4,700,000	.8	None	None	None	None	7,500	63%	2%	1%	2%	28%	1%	3%	0	0	0	0	0	260,000	25%
Symptomless	X	82%	4,600,000	.8	None	None	None	None	9,200	55%	1%	0	1%	39%	3%	1%	0	0	0	0	0	268,000	25%
Symptomless	XII	83%	4,800,000	.8	None	None	None	None	8,000	59%	2%	1%	1%	30%	3%	4%	0	0	0	0	0	253,000	25%
Symptomless	XIII	80%	4,700,000	.8	None	None	None	None	9,500	51%	1%	0	0	42%	1%	5%	0	0	0	0	0	300,000	25%
Mild.....	V	75%	4,000,000	.9	None	None	None	None	8,500	70%	1%	2%	0	21%	4%	2%	0	0	0	0	0	272,000	50%
Mild.....	VI	72%	3,800,000	.9	None	None	None	None	9,000	71%	2%	1%	1%	18%	2%	5%	0	0	0	0	0	280,000	50%
Mild.....	IX	60%	3,500,000	.8	None	None	None	None	10,000	60%	1%	1%	2%	32%	2%	2%	0	0	0	0	0	290,000	50%
Mild.....	XIV	65%	4,000,000	.8	None	None	None	None	9,000	70%	3%	1%	0	20%	2%	4%	0	0	0	0	0	300,000	50%
Severe	VII	40%	2,700,000	.7	Moderate	Moderate	Moderate Diffuse and Slight Punctate	3 Normoblasts	15,000	75%	3%	1%	0	15%	5%	1%	0	0	0	0	0	400,000	99%
Severe	XI	35%	2,800,000	.6	Moderate	Moderate	Moderate Diffuse and Slight Punctate	5 Normoblasts	12,000	60%	3%	1%	0	31%	1%	4%	0	0	0	0	0	370,000	99%

Symptomatology.—The following remarks are based on observations upon 20 cases. The symptoms may be (1) absent, (2) mild, (3) severe. Those that have no symptoms go through life as normal individuals. It is only by examination of the fresh blood that the diagnosis is made.

One is unlikely to see patients with mild symptoms, for the reason that their chief subjective symptoms, which are weakness and fatigue, endure for a brief period and disappear readily after rest in bed. The symptom periods occur infrequently in these cases and last from one to two weeks. Muscular and joint pains may occur in the mild form, but are more often present in the severe forms.

Since only patients with severe symptoms seek medical advise, these are the first to be recognized. Examination of other members of the family may bring to light the mild and symptomless forms. The chief symptoms in the severe cases are inability to work, due to weakness, leg ulcers, and muscular pain or stiffness, and often sharp pain in the epigastrium, especially after eating. The usual accompaniments of anæmia, such as shortness of breath on slight exertion, malaise, lassitude, swelling of the feet and ankles, dizziness and occasional headaches may be present. A slight injury or knock on the leg is often sufficient to provoke ulceration. Fever is usually present and may reach 101°F., but rarely goes higher unless associated with some superimposed infection such as tonsillitis to which these patients are usually susceptible. Morning remissions are the rule. In other cases the fever may be of low grade, never exceeding 99.5°F. (mouth). Night sweats are present in some of the cases. The long course constitutes a series of remission and relapses. In stages of remission the patients are able to do light work, their temperature may be normal and symptoms other than weakness or general illbeing, which they consider normal, are lacking. During the relapses, which last from three to six weeks, the weakness increases and the fever returns.

Physical Examination.—The scleræ are greenish yellow, the intensity varying with the period of relapse. The mucous membranes are pale: there is usually a slight general glandular enlargement and in many instances a slight pitting œdema of the ankles. The lungs are clear. The heart usually shows a slight enlargement to the left, with a soft systolic murmur heard over the whole precordium and sometimes transmitted to the left axillary line. The pulmonic second sound is accentuated. The blood pressure is low, varying from 100 to 105 systolic with a diastolic pressure of 55 to 70. The pulse is usually rapid, varying from 90 to 120 per minute and may exhibit marked hourly variations. Ranges of pulse from 85 to 110 per minute within two hours have been observed. The liver may be palpable, but not greatly enlarged. The spleen is not felt. The reflexes are active. The genitalia are infantile: axillary and pubic hair is scant. An ulcer may be found, or scars of old ulcers on the legs and feet may be present.

The urine shows a trace to a 2 plus albumin with a low fixed specific gravity 1010-1012. Urobilin is present. A few granular casts may be found. Phenolphthalein excretion is usually low, varying from 30% to 50% in two hours, showing a moderate grade of renal impairment. Gastric analysis shows usually an achlorhydria and in some cases a hypochlorhydria. No organic acids are found. The stools are negative for ova or parasites. The Was-

sermanns of the blood and spinal fluid are negative. Other spinal fluid examinations show no abnormalites. Blood cultures have been negative and cultures from the ulcers have shown the ordinary secondary invaders. The vital capacity varies between 20% and 50% below normal. Chemical examination of the blood shows nothing unusual. The total serum protein, calcium, and inorganic phosphorus are within normal limits.

Diagnosis.—The disease may be suspected after superficial examination. The blood picture is sufficiently characteristic, the anæmia with the presence of sickle or crescent-shaped cells, and the characteristic sickling of all cells in the fresh wet preparations after 24 hours. Syphilis, tuberculosis, and hæmolytic anæmias should be eliminated by appropriate examinations.

Prognosis.—In the symptomless and mild cases the prognosis is excellent, but in the severe forms it is distinctly unfavorable on account of the susceptibility of these patients to intercurrent infections, especially pneumonia and tonsillitis. They seldom live beyond the age of 30 years.

Treatment.—The treatment consists of simple hygienic measure with amplified diet and rest in bed. Medicinal therapy consists of the administration of iron in adequate doses, preferably in the form of Blaud's pills (soft mass) 5 grs. t.i.d. p.c. Arsenic may be given in the form of Fowler's solution or intramuscularly in the form of sodium cacodylate. The ulcers, if present, should be treated with an ointment of boric acid or zinc oxide. The disease has never been cured, but with treatment and rest, the relapses are shortened and the remissions are made longer. The patients should never be allowed to perform hard physical work.

Conclusions

1. Sickle cell anæmia is a specific disease entity, described thus far only in the negro race.
2. The disease is fairly common.
3. The sickling of the red blood cells is due to something inherent within the cells; not to any substance in the serum. This is possibly a surface tension phenomenon, not occurring in the circulating blood to any marked degree.
4. It is transmitted according to the Mendelian law.
5. Attempts to transmit the disease to animals have thus far been unsuccessful.

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A CLINICAL EPIDEMIOLOGICAL STUDY OF ACUTE TONSILLITIS AND ACUTE UPPER RESPIRATORY INFECTIONS*

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This communication is part of a study on streptococcus infections with especial reference to acute tonsillitis. The present report deals with clinical epidemiological features. An attempt was made to obtain information on the following points. (1) Is acute tonsillitis a seasonal disease? (2) If seasonal, can any special epidemiological factors be demonstrated? (3) Can any direct spread be traced from case to case in an outbreak? At the same time association of tonsillitis with upper respiratory tract infections was studied.

The great majority of the cases of acute tonsillitis seen in The Johns Hopkins Hospital occur among the nurses, partly because this group is a large fraction of the personel of the institution, and partly because few patients from the outside seek admission with this disease. This group is a very favorable one to study epidemiologically, for it is, to a great extent, isolated from the surrounding community, the individuals live in close contact with one another, and their environment remains constant throughout the year. Besides, they can be closely and frequently observed.

To some of us who have been keenly interested in acute tonsillitis for several years, it seemed that the majority of the cases occurred during the winter months, and that the disease was quite rare in summer. In order to be sure of this seasonal variation, the records of the past four years were consulted. From April 1, 1919 to March 31, 1923 there occurred 141 cases of acute tonsillitis among the pupil nurses of The Johns Hopkins Hospital Training School. Chart 1 shows the total number of cases for the past four years grouped according to months. From this it can be seen that the incidence of the disease increases during October, remains high during November, December, January, February and March, falls in April, and remains low during the summer. On

* This is the third of a series of papers on streptococcus infection with special reference to acute tonsillitis.

the same chart is placed a curve representing the total number of cases of scarlet fever in the city of Baltimore reported to the Board of Health during the last three years. These cases are also grouped according to months. The comparison is made between the two diseases because of the constant presence of the hemolytic streptococcus in the throats of scarlet fever patients, as well as in the throats of those suffering from acute tonsillitis. From these figures the conclusion that acute tonsillitis is a seasonal disease, occurring in the late fall, winter and early spring, seems justified.

The next step was to determine whether any special epidemiological factors could be demonstrated. For this study 194 pupil nurses were selected. The investigation began September 1, 1922, and ended March 31, 1923. This group consisted of nurses five of whom had been in training for 3 years, thirteen for 2½ years, forty-three for 2 years, twelve for 1½ years, fifty-seven for 1 year, twenty for 6 months and thirty-seven for 1 month. Seven were taken from a number who entered during the study. During the month of September, before the first case of tonsillitis occurred, the individuals were interviewed and their room numbers and the names of their room-mates were obtained. The object of the study was explained and they were asked to report promptly any respiratory infection, even though slight. A daily visit was made to the nurses home to receive these reports and to examine the cases. The co-operation of the pupil nurses, as well as of the superintendents, was excellent, and we have reason to believe that the great majority of the respiratory infections were reported. When a case of tonsillitis was discovered, a very careful investigation was made in order to trace, if possible, the source of the infection. The patients were questioned regarding the health of their room-mates, contact with patients in the wards suffering from respiratory infections, and contact with persons who had had the disease previously.

From September 1, 1922 to March 31, 1923 there occurred in this group 42 cases of acute tonsillitis, 53 colds, 44 cases of acute pharyngitis, 11 cases of acute laryngitis, 28 cases of influenza and 9 cases of atypical acute upper respiratory infections which could not be placed in any of the foregoing groups. Of the cases of tonsillitis 2 occurred in September, 7 in October, 6 in November, 2 in December, 11 in January, 11 in February and 3 in March. This incidence coincides fairly closely with the incidence curve for the preceding three years. Chart 2 shows the acute upper respiratory infections which occurred during this period. Each case is represented by a black square, and where more than one case of the same disease occurred on the same day, the squares are placed one above the other.

The cases of tonsillitis were then analyzed to determine, if possible, any existing relation between the occurrence of the disease and the weather. Chart 2 shows the

TABLE I.

This table shows the range in the relative humidity per cent, hours of actual sunshine, precipitation and the number of cases of tonsillitis.

Date	Relative humidity per cent		Precipitation	Hours of actual sunshine	Cases of Tonsillitis
	Max.	Min.			
Sept. 1	90	65		4.3	
Sept. 2	95	81	.61	1.5	
Sept. 3	89	75		6.7	
Sept. 4	92	62		9.1	
Sept. 5	86	59		10.7	
Sept. 6	68	42		12.8	
Sept. 7	85	63		10.6	
Sept. 8	88	82	.92	0.0	
Sept. 9	90	80		5.5	
Sept. 10	91	60		9.3	
Sept. 11	90	69		6.3	
Sept. 12	86	62	1.16	7.5	
Sept. 13	64	42		12.5	
Sept. 14	76	45		12.5	
Sept. 15	89	65		10.4	
Sept. 16	70	44		12.4	1
Sept. 17	59	41		9.6	
Sept. 18	64	50		10.9	
Sept. 19	76	56		6.1	
Sept. 20	86	60		7.2	
Sept. 21	79	53		9.	
Sept. 22	66	51		8.	
Sept. 23	69	53		11.	
Sept. 24	75	38		8.	
Sept. 25	58	37		12.	
Sept. 26	55	40		12.	
Sept. 27	72	48		12.	
Sept. 28	83	48		11.9	
Sept. 29	88	82		4.5	
Sept. 30	100	84		8.8	
Oct. 1	90	66		11.8	1
Oct. 2	69	46		11.6	
Oct. 3	65	35		11.1	
Oct. 4	71	58		10.9	1
Oct. 5	63	31		11.6	2
Oct. 6	63	32		6	
Oct. 7	91	76	.12	0.0	
Oct. 8	85	61	.05	6.1	2
Oct. 9	95	70	.22	3.4	
Oct. 10	91	89	5.08	0.0	
Oct. 11	75	41		9.4	
Oct. 12	69	36		10.7	1
Oct. 13	59	39		11.3	
Oct. 14	73	55		3.7	
Oct. 15	88	66	.01	5.9	
Oct. 16	88	68		6.3	1
Oct. 17	85	32		7	

TABLE I.—Continued

Date	Relative humidity per cent		Precipitation	Hours of actual sunshine	Cases of Tonsillitis
	Max.	Min.			
Oct. 18	58	30		11.1	
Oct. 19	72	51		11.	
Oct. 20	66	45		11.	
Oct. 21	72	53		9.8	
Oct. 22	86	64		8.5	
Oct. 23	91	41	0.1	2.7	
Oct. 24	63	46		10.	
Oct. 25	78	40		8.1	
Oct. 26	58	49		7.3	
Oct. 27	60	40		10.3	
Oct. 28	60	40		10.3	
Oct. 29	64	43		10.2	
Oct. 30	61	41		10.6	
Oct. 31	74	44		10.6	
Nov. 1	72	55		10.5	
Nov. 2	98	63	.02	3.3	
Nov. 3	77	59		4.	
Nov. 4	83	70		0.0	
Nov. 5	81	70		6.4	
Nov. 6	98	84		3.8	
Nov. 7	99	89	.17	1.8	
Nov. 8	76	39		9.4	
Nov. 9	61	51		9.1	
Nov. 10	70	38		10.2	
Nov. 11	88	47		10.1	1
Nov. 12	64	43		7.4	
Nov. 13	62	29		7.7	1
Nov. 14	68	47		5.6	
Nov. 15	81	65	.23	0.7	
Nov. 16	52	45		8.6	
Nov. 17	52	45		10.	
Nov. 18	83	59	.03	2.5	
Nov. 19	86	51		9.9	
Nov. 20	58	48		7.4	
Nov. 21	54	47		7.5	1
Nov. 22	69	52		2.4	1
Nov. 23	60	45		9.3	
Nov. 24	74	40		7.	1
Nov. 25	53	47		4.1	
Nov. 26	56	47		5.4	
Nov. 27	72	50		0.0	
Nov. 28	76	66		3.8	
Nov. 29	67	52		9.7	1
Nov. 30	78	34		8.9	
Dec. 1	65	50		1.1	
Dec. 2	54	42		8.7	
Dec. 3	88	78		3.3	
Dec. 4	86	72	0.7	0.2	1
Dec. 5	96	34	2.6	6.9	
Dec. 6	50	45		9.6	

TABLE I.—Continued

Date	Relative humidity per cent		Precipitation	Hours of actual sunshine	Cases of Tonsillitis
	Max.	Min.			
Dec. 7	98	68	.11	0.0	1
Dec. 8	100		.12	0.0	
Dec. 9	56	52		4.4	
Dec. 10	82	65	.26	7.3	
Dec. 11	84	64		3.6	
Dec. 12	90	33		0.0	
Dec. 13	65	43		1.6	
Dec. 14	95	56	.13	0.0	
Dec. 15	90	61	.15	0.0	
Dec. 16	66	60	.01	5.1	
Dec. 17	98	71	.75	0.0	
Dec. 18	59	39		7.2	
Dec. 19	70	46		9.0	
Dec. 20	79	57		7.2	
Dec. 21	78	50	.01	5.4	
Dec. 22	66	63		4.2	
Dec. 23	68	71		2.0	
Dec. 24	91	77		5.0	
Dec. 25	100	73		2.7	
Dec. 26	100	83		5.7	
Dec. 27	100	48	.14	0.0	
Dec. 28	98	87	1.04	0.0	
Dec. 29	78	47		9.1	
Dec. 30	67	62		7.9	
Dec. 31	89	88		3.2	
Jan. 1	92	66	.76	3.7	1
Jan. 2	76	55		7.2	
Jan. 3	93	79	.1	0.0	
Jan. 4	75	65	.1	4.1	1
Jan. 5	73	55		2.9	3
Jan. 6	82	68		3.9	
Jan. 7	95	81		0.0	
Jan. 8	93	67	1.04	3.1	
Jan. 9	85	56	.17	0.4	
Jan. 10	60	55		0.9	
Jan. 11	69	53		7.	1
Jan. 12	73	41		7.8	
Jan. 13	62	49		9.7	
Jan. 14	86	47	.02	1.5	
Jan. 15	65	55	.01	0.3	
Jan. 16	54	45		7.2	1
Jan. 17	50	18		9.8	
Jan. 18	52	30		3.6	
Jan. 19	54	36		7.8	
Jan. 20	90	75		0.0	
Jan. 21	82	65		2.9	1
Jan. 22	80	61	.15	3.8	1
Jan. 23	62	41		6.0	
Jan. 24	100	85	1.1	0.0	1
Jan. 25	64	54		7.4	

TABLE I.—Continued

Date	Relative humidity per cent		Precipitation	Hours of actual sunshine	Cases of Tonsillitis
	Max.	Min.			
Jan. 26	75	59		5.3	
Jan. 27	93	70	.05	0.0	
Jan. 28	97	83	.5	0.0	1
Jan. 29	58	55		6.4	
Jan. 30	72	51		8.8	
Jan. 31	95	66		0.0	
Feb. 1	97	94	.42	0.0	1
Feb. 2	90	78	.01	0.0	
Feb. 3	97	61	.03	0.0	1
Feb. 4	66	54		0.5	
Feb. 5	53	41		0.3	
Feb. 6	78	49	.16	0.0	
Feb. 7	82	61	.07	6.3	
Feb. 8	83	60		6.0	
Feb. 9	84	54		7.2	
Feb. 10	89	53	.03	3.2	
Feb. 11	90	51	.03	6.7	
Feb. 12	90	42	.01	6.7	
Feb. 13	100	87	1.45	0.0	
Feb. 14	48	23	.08	10.7	
Feb. 15	48	34		10.8	
Feb. 16	48	20		10.8	
Feb. 17	66	43		1.6	1
Feb. 18	44	28		10.9	
Feb. 19	54	40		9.6	
Feb. 20	89	82	.08	3.5	
Feb. 21	70	58		11.0	1
Feb. 22	63	50		6.5	
Feb. 23	50	33		8.6	1
Feb. 24	75	33		9.6	2
Feb. 25	60	42		5.5	1
Feb. 26	97	64	.04	0.0	1
Feb. 27	98	82	.23	0.0	1
Feb. 28	85	50		5.8	1
Mar. 1	67	58		4.8	
Mar. 2	66	38		8.9	
Mar. 3	49	31		9.5	1
Mar. 4	64	32		7.6	
Mar. 5	57	50		0.4	
Mar. 6	95	91	.83	0	
Mar. 7	76	52	.66	9.1	1
Mar. 8	56	49		10.8	1
Mar. 9	60	42		11.7	
Mar. 10	79	51	.02	1.9	
Mar. 11	65	42		8.7	
Mar. 12	100	56	.22	3.7	
Mar. 13	88	84	.70	0	
Mar. 14	79	51		9.3	
Mar. 15	76	57		10.5	
Mar. 16	91	81	.76	0	

TABLE I.—Continued

Date	Relative humidity per cent		Precipitation	Hours of actual sunshine	Cases of Tonsillitis
	Max.	Min.			
Mar. 17	66	45	.21	10.4	
Mar. 18	79	48		12.1	
Mar. 19	96	47	.4	0	
Mar. 20	63	30		12.0	
Mar. 21	70	38		12.2	
Mar. 22	79	52		5.0	
Mar. 23	89	55	.32	6.5	
Mar. 24	49	38		11.5	
Mar. 25	55	39		11.0	
Mar. 26	72	41		7.3	
Mar. 27	76	33		12.4	
Mar. 28	34	30		11.6	
Mar. 29	62	36		12.5	
Mar. 30	54	27		8.8	
Mar. 31	34	19		12.1	

normal mean temperature, and the maximum and minimum daily temperatures in Baltimore during this period of seven months. Table I shows the daily range in the relative humidity per cent, hours of actual sunshine, and daily precipitation. As the incubation period of acute tonsillitis is probably a short one, the weather on the preceding three days as well as on the day of the onset of the disease was studied in relation to each case.

Variations of the Daily Mean Temperature Above or Below the Normal Mean Temperature.—In 15 cases the mean temperature was below and in 24 cases above the normal on the day of onset. For the day before onset, in 13 cases the mean was below and in 26 cases above normal. For the second day before the onset, in 13 cases the mean was below and in 26 cases above normal. For the third day before onset in 13 cases the mean was below and in 25 cases above normal. This shows that abnormally cold weather did not predispose to tonsillitis.

Variations in the Daily Temperature.—The days during this period were divided into those during which the temperature varied 15° or less and into those during which the temperature varied 16° or more (Table II).

TABLE II.

Days with variations in temperature of 15° or less			
Day of onset 29 cases	1 day before 30 cases	2 days before 29 cases	3 days before 22 cases
Days with variations in temperature of 16° or more			
Day of onset 13 cases	1 day before 12 cases	2 days before 13 cases	3 days before 20 cases

From this we can say that great variations in daily temperature did not predispose to tonsillitis.

Relative Humidity Per Cent.—The days during this period were divided into those on which the maximum

humidity was above the average and into those on which the maximum humidity was below the average. The average maximum humidity was 76% (Table III).

TABLE III.

Days on which the maximum humidity was above the average.			
Day of onset	1 day before	2 days before	3 days before
17 cases	17 cases	15 cases	13 cases
Days on which the maximum humidity was below the average.			
Day of onset	1 day before	2 days before	3 days before
25 cases	25 cases	27 cases	29 cases

The days were likewise divided into those on which the minimum humidity was above the average and into those on which the minimum humidity was below the average. The average was 53% (Table IV).

TABLE IV.

Days on which the minimum humidity was above the average.			
Day of onset	1 day before	2 days before	3 days before
21 cases	24 cases	17 cases	15 cases
Days on which the minimum humidity was below the average.			
Day of onset	1 day before	2 days before	3 days before
21 cases	18 cases	25 cases	27 cases

From these figures it can be seen that no relation existed between the relative humidity and the occurrence of acute tonsillitis.

Cloudy Weather.—Certainly if the sun shines for 7 hours during any day from September 1st to March 31st, that day cannot be considered a cloudy one. The days were divided into those during which the sun shone for 7 hours or more and into those during which it shone less than 7 hours (Table V).

TABLE V.

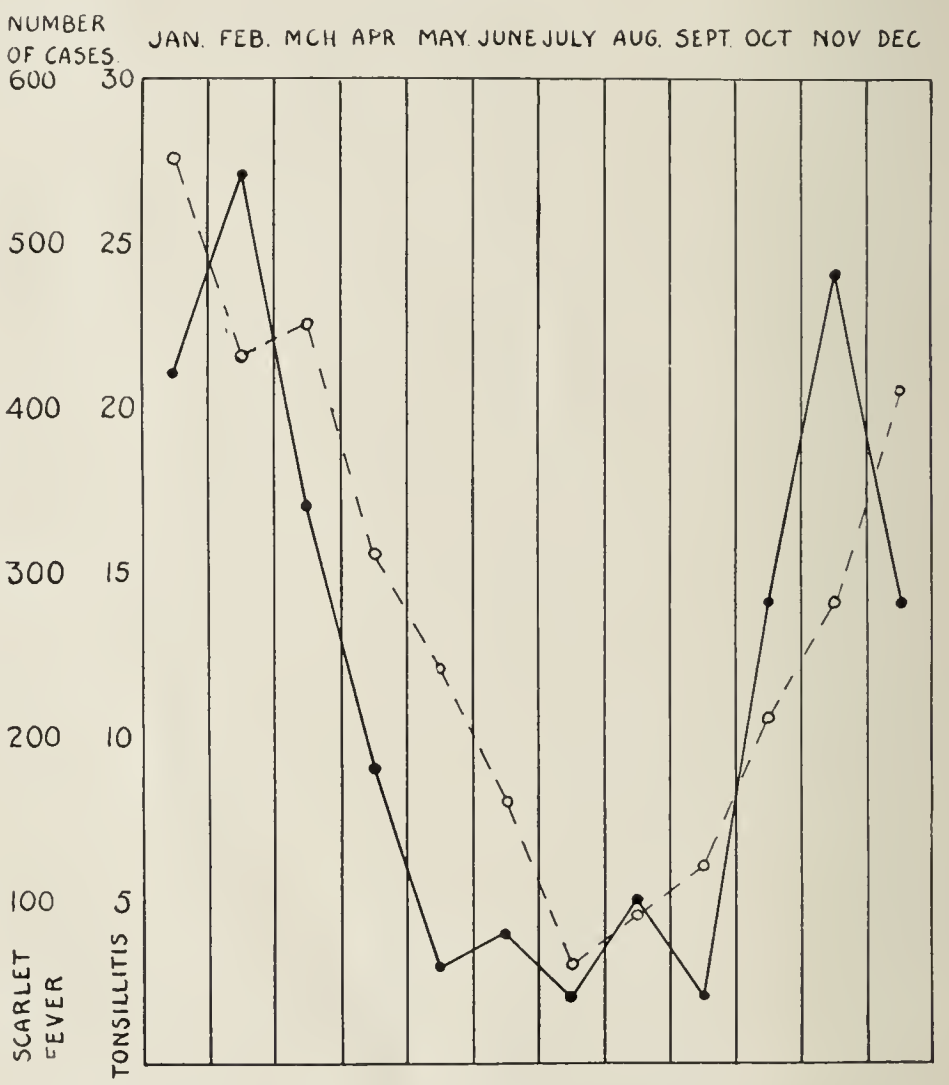
Days during which the sun shone 7 hours or more.			
Day of onset	1 day before	2 days before	3 days before
17 cases	17 cases	19 cases	26 cases
Days during which the sun shone less than 7 hours.			
Day of onset	1 day before	2 days before	3 days before
22 cases	22 cases	20 cases	13 cases

These figures show that no relation existed between the hours of sunshine and the occurrence of acute tonsillitis.

Rainfall.—In fifteen of the forty two cases it did not rain either on the day of the onset or on any of the preceding three days. In the remaining twenty-seven cases there was rain on one or more of these four days. This is a ratio of about 1 to 3. There were only 69 in this period of 212 days when the combination of four clear days in succession occurred, a ratio also of about 1 to 3. Therefore, no relation could be demonstrated between rainfall and acute tonsillitis in this series of cases.

From Chart 2 it can be seen that the cases of acute tonsillitis occurred more or less in groups. During September there was only one case, but during the first sixteen days of October eight cases occurred. Following this there was a period of twenty-seven days without a case, then eight cases in twenty-seven days. The last twenty-four days of December were without cases. During January, February and the first week in March, the cases were fairly evenly distributed, except for the last eight days in February when eight cases occurred. The occurrence of colds and acute pharyngitis ran parallel with that of acute tonsillitis. There was an increase in the number of cases during the latter part of September and the first half of October with a decrease in the latter part of October and the early part of November. This was followed by a rise in November with a fall in December and a prolonged rise in January, February and the first half of March. This was not true of influenza. The first case was seen on the third of January and the majority of the cases, 25 out of a total of 28, occurred between January 21, and February 22.

CHART 1.



Acute tonsillitis is represented by the solid line and scarlet fever by the broken line.

It was almost impossible to trace the direct spread of tonsillitis from case to case. Of 194 nurses 64 roomed alone, 126 roomed in pairs and 4 lived together in one large room. Twelve cases of tonsillitis occurred in those rooming alone and 30 cases occurred in the remainder. The disease occurred in both room-mates in only two instances. In the first instance there was an interval of

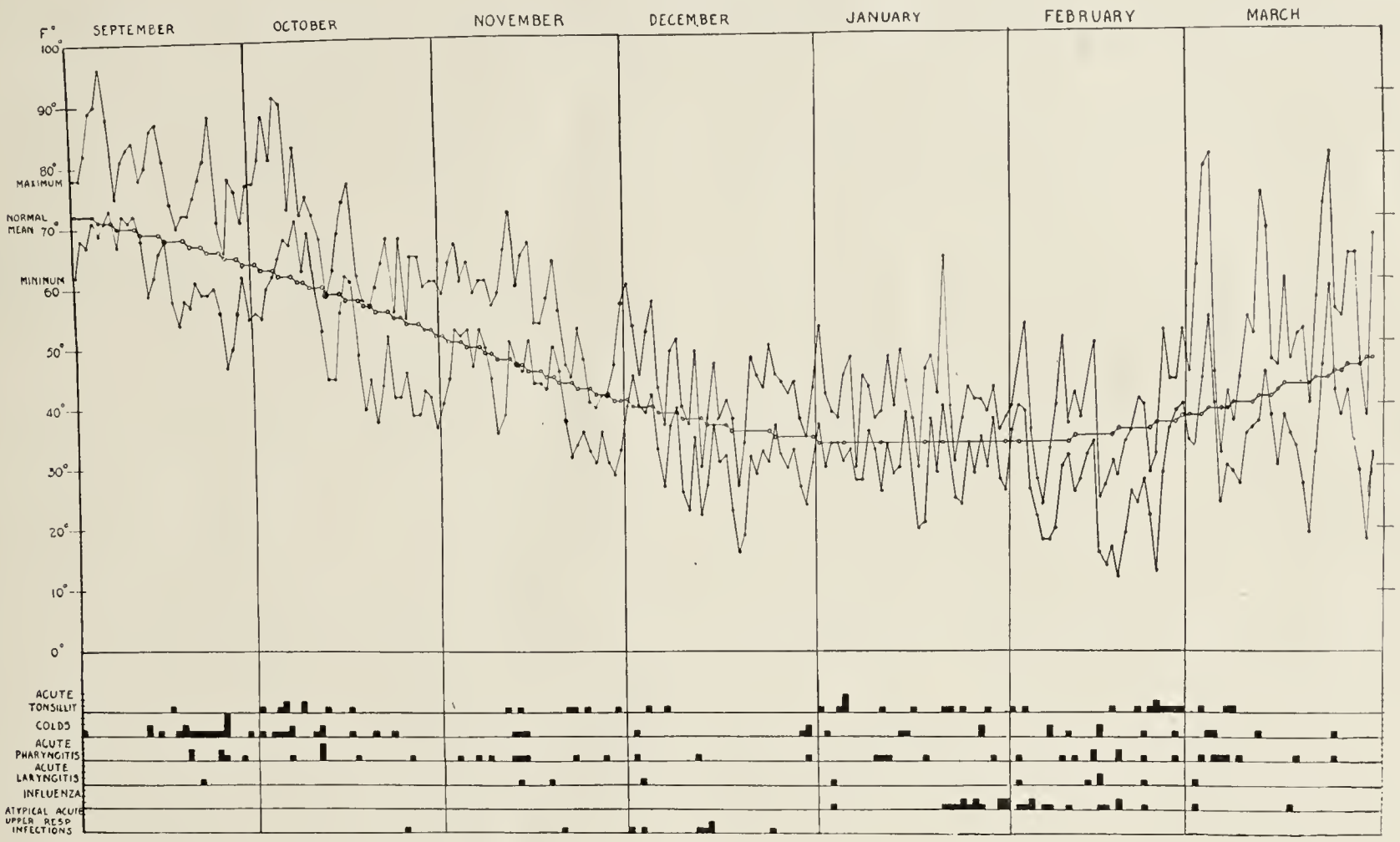
15 days between the onset of the first case and that of the second, but 4 days after Patient 1 returned to her room, Patient 2 came down with the disease. In the second instance 106 days intervened between the two cases. When all other possible sources of infection are studied, we find that in 33 cases no history of any contact was obtained. In only 8 cases was it possible even to speculate on the transmission of the disease from one person to another. However, one nurse, while caring for a case of tonsillitis in the ward, came down with the disease.

Conclusions

A clinical epidemiological study of acute tonsillitis was undertaken to obtain information on the following points. 1. Seasonal variation. 2. The significance of possible epidemiological factors, such as weather and the association with other acute upper respiratory infections. 3. The direct spread of the disease from patient to patient. It was found that tonsillitis was seasonal in its occurrence, that there was no relation demonstrable between weather conditions and the disease, and that the occurrence of acute tonsillitis, colds and acute pharyngitis, ran parallel. It was very difficult to trace the direct spread of the disease from case to case.

I wish to thank the pupil nurses for their co-operation and the superintendents of nurses for their aid in this study.

CHART 2.



SUPERVENTILATION AND CARBON-DIOXIDE ELIMINATION

John T. King, Jr.

With the Technical Assistance of

Virginia B. Cross

(From the Medical Clinic of the Johns Hopkins Hospital)

In a recent report[1] I made a critical analysis of the standard observations of DuBois upon cases of thyroid disease. It was found that oxygen absorption gave, in this series of cases, an excessively high index to the heat production even after careful preparation of the patients for the test. The high O_2 intake caused the total indirect calorimetry to be elevated above direct calorimetry. CO_2 elimination was found to afford the best caloric index when collected under the ideal conditions of the chamber calorimeter.

The "Tissot" method is so well established that we hesitate to make comparisons of the value of a single gas determination, such as CO_2 elimination, with it. It was

[1] King, J. T. Jr.: The Gas Exchange in Diseases of the Thyroid Gland. Johns Hopkins Hosp. Bull., 1923, XXXIV, 304.

shown in the above analysis, however, that a more accurate prediction can be made of the heat production from the carbon-dioxide figures alone than is possible from the total indirect calorimetry. This fact is brought out not to depreciate the "Tissot" method in practice but to show why I feel that a serious attempt should be made to perfect a method for the collection of CO_2 from the expired air, a method for the routine determination of the basal metabolic rate.

There has always been the disadvantage in CO_2 collection that subjects may cause an excessive elimination of this gas through mere superventilation of the lungs. That CO_2 may be washed out of the blood through hyperpnœa is so well established and the literature upon this subject is so abundant, that it seems unnecessary to discuss it here. It has always raised the question whether CO_2 elimination can offer a safe measure of the heat production of the body.

The process of compensatory carbon-dioxide retention following the washing out through forced breathing has been studied by Boothby.[2] This observer studied two subjects; one of these reacted in the usual way by passing into a state of apnœa following two minutes of forced breathing; the other subject passed from two minutes of forced breathing through the usual short phase of hyperpnœa directly into natural breathing without any period of apnœa. The CO_2 elimination behaved in the case of the second subject just as it did in the case of the first: it was retained after the excessive elimination due to superventilation. In both cases the body had retained, at the end of 15 minutes, nearly although not quite as much as had been lost through the forced breathing. Boothby's conclusion was that, in spite of the absence of apnœa in some individuals, the loss of carbon-dioxide through forced breathing is made up within a few minutes, though not so rapidly as when apnœa occurs. He also felt that there must be some factor other than apnœa at work to account for this and believed that there may occur a slowing of the circulation to aid in the process of retention. It is interesting to note that, while the ventilation of the lungs was increased a total of about 60 liters in the 15-minute period, the total increase in CO_2 elimination for this period amounted to only about 200 c.c.

While the total ventilation of the lungs in Boothby's experiments was greatly increased, they furnish us only with information as to what happens when the forced breathing occurs at the very beginning of the period of observation.

As far back as 1866 and 1870 Lossen [3,4] studied the CO_2 elimination for fifteen-minute periods, increasing the ventilation of the lungs from 75.1 liters to 182.7 liters for the period in question. He was unable to demonstrate any increase of CO_2 elimination other than what might be expected from the excessive use of the respiratory muscles. The determination of CO_2 elimination in Lossen's experiments was made by an analysis of the CO_2 percentage in the expired air, which passed through a two-liter flask, the contents of which were analyzed for CO_2 at the end of each experiment. The value of this work lies not, as Lossen then supposed, in demonstrating the CO_2 output for 15 minutes, but it did show the percentage of CO_2 in the expired air at the end of various degrees of superventilation for 15 minutes.

In order to obtain some data upon the manner in which CO_2 is lost from the blood under superventilation a series of experiments was carried out upon myself as a subject, with the aid of Miss Cross. The method of these experiments is illustrated in Figure 6 on insert plate.

Method

The meter used to measure the intake of air is a laboratory test meter of the "wet" type, which was kindly lent us for this work by the Consolidated Gas and Electric Company of Baltimore. This type of meter has been studied by the U. S. Bureau of Standards [5] and found to be subject to not more than 0.2% of error, with reasonable care in adjusting the water level in the meter. The meter delivers about 0.5% more air when the air passes through at a rate of 20 cubic feet per hour and it delivers about 1.0% more air when it passes at a rate of 30 cubic feet per hour. Inasmuch as the air passed through the meter only during inspiration in our tests, the total rate of flow for any period was considered to be approximately twice as fast as the total amount of air delivered for a given period. In no case was the rate of flow on this basis more rapid than 30 cubic feet per hour. The volume of air delivered was corrected for the rate of flow, and reduced to 0° C. and 760 mm. Hg. barometric pressure. A series of experiments with these meters [5] showed that gas which entered the meters in an unsaturated condition of humidity left them practically saturated at the temperature of the meter. Since our interest in these observations consists entirely in comparative air volumes, the correction for aqueous tension was not made. Temperature readings were made for each period from the thermometer in the air chamber of the meter. The technic of the test is the same as that described by me [6] for the collection of CO_2 elimination by passing the expired air through a series of three jars—calcium chloride, soda lime and calcium chloride again, after the model of the CO_2 collecting part of the Benedict portable apparatus. Inspired air from a freshly ventilated room came directly through the meter and passed by air-tight tubing to the subject.

All the tests were made upon myself, aged 33 years, body surface—1.85 sq. m., during the late morning hours after a uniform breakfast of one egg and one slice of toast at least two

[2] Boothby, W. M.: Absence of Apnœa After Forced Breathing. Jour. Phys., 1912-1913, XLV, 328.

[3] Lossen, von. H.: Über den Einfluss der Zahl und Tiefe der Athembewegungen auf die Auscheidung der Kohlensäure durch die Lungen. Ztschr. f. Biologie, 1866, II, 244.

[4] *Idem:* (Continuation of 3). *Ibidem*, 1870, VI, 298.

[5] Waidner, C. W. and Mueller, E. F.: Technologic Papers of the Bureau of Standards. No. 36. Industrial Gas Calorimetry. Washingtion. 1914.

[6] King, J. T., Jr.: Determination of the Basal Metabolism from the Carbon-Dioxide Elimination. Johns Hopkins Hospital Bull., 1921, XXXII, 277.

hours before. A rest period of at least twenty minutes was observed in each case. In each test a uniform rate of ventilation was maintained with the aid of a bell on the meter, which struck at the end of the passage of each 1/10 cubic foot of air.

Observation 1

This experiment was made to test the validity of the observations of Lossen, who found that superventilation of the lungs caused no increase of CO_2 elimination if collected over a 15-minute period. The first period was occupied with natural breathing (volume 57.52 liters); this ventilation was almost doubled in the second period (volume 102.2 liters). This superventilation was accomplished with considerable effort on the part of the subject and the rate of respiration was maintained at as nearly uniform a rate as possible.

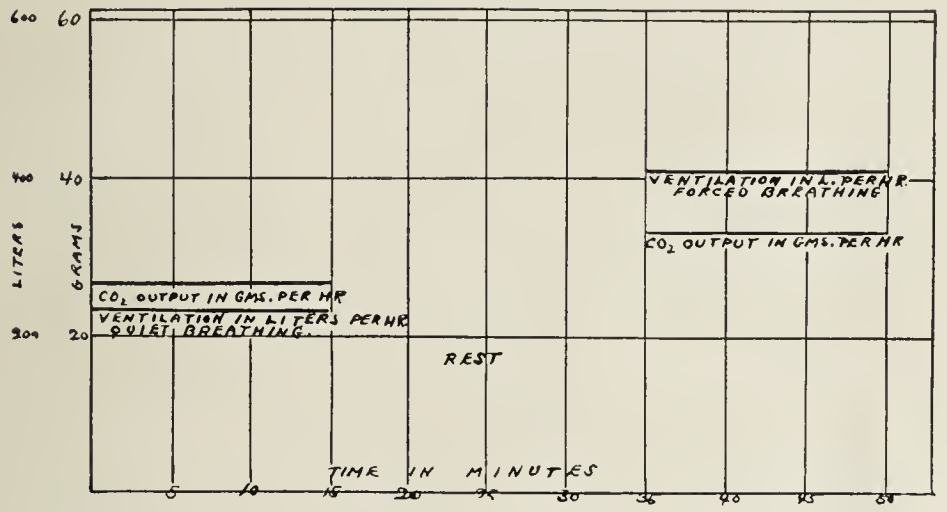


Fig. 1.—Showing effect of superventilation on CO_2 output.

The effect of superventilation at a uniform rate for 15 minutes is, therefore, very striking in the increase of CO_2 elimination. The ventilation was increased about 77% in the 2nd period, and the CO_2 output was raised about 28%. The results differ from Boothby's observations because in his cases the superventilation was limited to the first 2 minutes of the fifteen-minute period, which was followed by a period of natural breathing with CO_2 retention. The result also differs from those of Lossen.

Observation 2

Lossen attributed the slight increase in CO_2 output, which he obtained by superventilating the lungs, to the excessive use of the muscles of respiration. In our Observation 1 (Fig. 1) it was noted that the rate of respiration was 11 per minute during the period of quiet breathing and 25 per minute during forced breathing. The respiratory muscles were, therefore, exerted 14 times more per minute during the second period. It is obvious that such muscular activity must add somewhat to the CO_2 formation and elimination, and we wished to know how much additional CO_2 could be traced to such an origin. Observation 2 was carried out to test this point.

It was impossible artificially to bring the respiratory muscles into play without affecting the respiration, and the abdominal muscles were used as a substitute. Between natural respirations, therefore, I contracted my

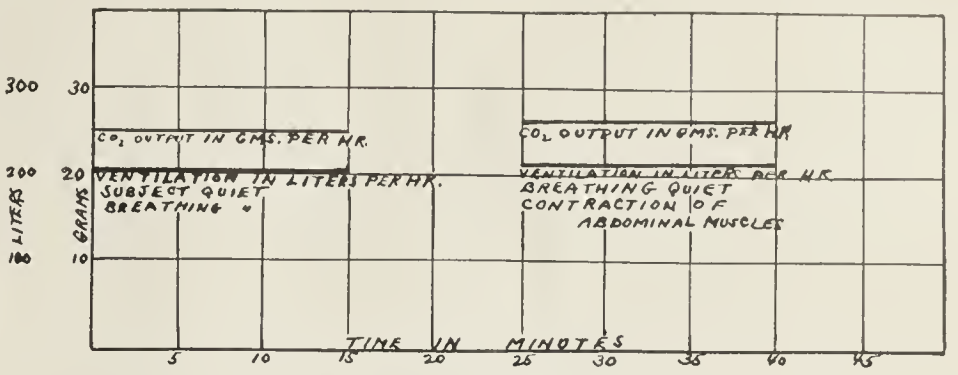


Fig. 2.—Effect of muscular activity. (See text.)

abdominal muscles vigorously at a rate of 14 times per minute—an effort which I consider at least equal to a sharp respiration. As a result of such activity the rate of ventilation of the lungs was increased by 8.52 liters per hour and the CO_2 output was elevated 1.00 gm. per hour, or 4%. We feel sure, therefore, that only about 4% of the increased CO_2 output from superventilation in Observation 1 can be attributed to muscular activity and that the remaining 24% must be the result of a release of excess CO_2 from the blood.

Observation 3

Observation 3 was done to test the effect of marked superventilation over two consecutive 10-minute periods. The conditions of this test, except for the light breakfast noted above, were exactly those of a routine basal metabolism test. Superventilation was comparatively easy to carry out in the first period but, in spite of a fifteen-minute rest interval, it was found impossible to continue the rate of ventilation through a second period, owing to the discomfort encountered.

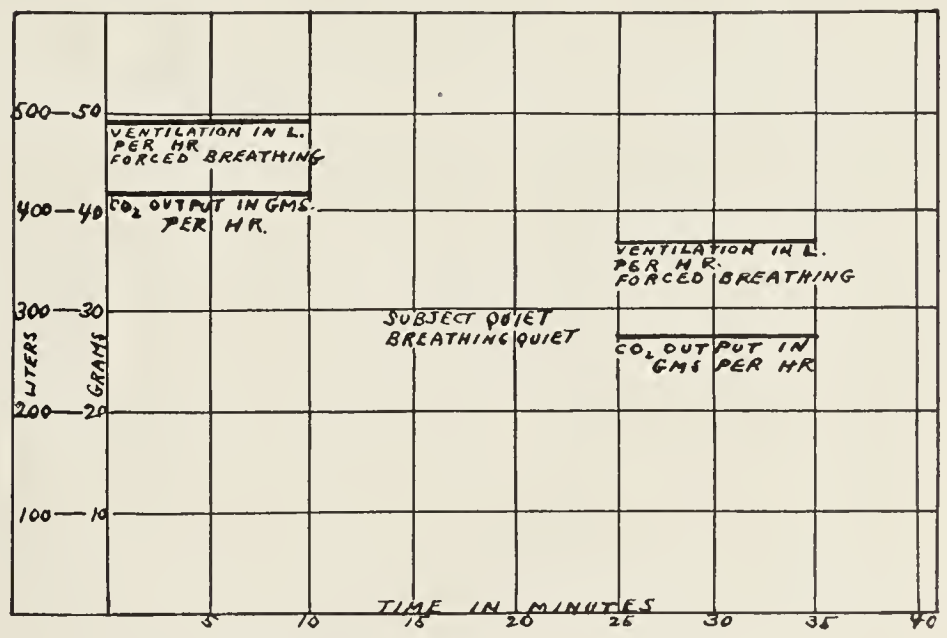


Fig. 3.—Consecutive periods of superventilation.

The attempt to persist in superventilation of the lungs is at least as difficult as the continuance of running after the onset of dyspnœa. In spite of the 15-minute rest between periods, it will be seen that the CO_2 output in the second period was elevated only some 5 or 10% above normal, while the rate of ventilation was nearly twice

that of quiet breathing. In this case it is probable that practically all the detachable CO_2 was "washed out" during the first period, as there is little more CO_2 elevation in the second period than might be the result of muscular activity. It is also interesting to note that the 15-minute rest period did not restore the combined CO_2 of the blood. It is presumed that the blood alkali reserve was shifted into the tissues or eliminated in the urine after the dissociation from it of the fixed CO_2 in some such way as has been observed by Haggard and Henderson.[7] If the buffer salts of the blood remained unchanged, we should expect a reaccumulation of combined CO_2 after 15 minutes, but this is apparently not the case.

From the practical standpoint this experiment helps us by showing what would be obtained in the case of a patient who breathes excessively in two consecutive periods of a basal metabolism determination; under these circumstances we should fail to get any kind of check between the two periods.

Observation 4

This experiment was done to determine the result of a moderate superventilation, persisted in at a perfectly even rate of ventilation. The first period is one of quiet breathing under basal conditions. In the second period the ventilation was increased 24% and the CO_2 rose 21.4%. In the third period the ventilation was maintained at the same level and the CO_2 output was 16% above normal. The maintenance of this degree of superventilation became difficult early in the last period and was held in spite of a constant desire on the part of the subject toward apnœa.

From this case it becomes obvious that a "check" of a little over 5% may be obtained between two periods of superventilation, provided the respiration be maintained perfectly constant.

Having myself been the subject, however, it is my belief that a patient, under such circumstances would have lessened the rate of ventilation in the last period, owing to the great physiologic demand for relief through apnœa. It marks a condition, however, which must be borne in mind as a theoretical possibility in carrying out metabolism determinations by the CO_2 method.

[7] Haggard, H. W. and Henderson, Y.: Haemato—Respiratory Functions. IV. How Oxygen Deficiency Lowers Blood Alkali. Jour. Biol. Chem., 1920, XLIII, 15.

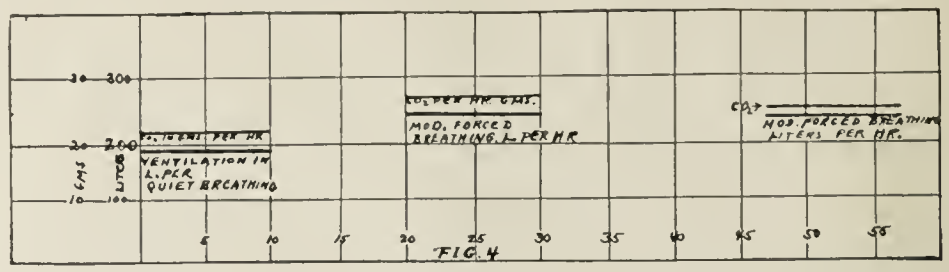


Fig. 4.

Observation 5

It was thought advisable to carry out a series of observations to show the effect of several periods of moderate superventilation.

In figure 5 is shown: first, a period of natural breathing under basal conditions except the routine breakfast; the last three periods show the effect of an increase of ventilation that amounted to 55%, 60% and 57%. The CO_2 elevation for these three periods was 45%, 23%, and 18%, respectively. The persistent superventilation was quite disagreeable in the last two periods.

Experiment A is similar to nos. 1 and 2, showing the effect of moderate superventilation for 15 minutes, following quiet breathing. There was a difference of a little more than 16% between the two periods.

Such observations as the foregoing help us to see what would happen under certain conditions in the carrying out of basal metabolic rate determinations by the CO_2 method. We see that CO_2 elimination is readily elevated by superventilation, but that in repeated periods of marked superventilation the CO_2 output fails to "check" within reasonable limits. In Figure 5, however, it is seen that after continued marked superventilation the CO_2 output drops in the last two periods and checks within 5%. This follows two periods that differed by 22%.

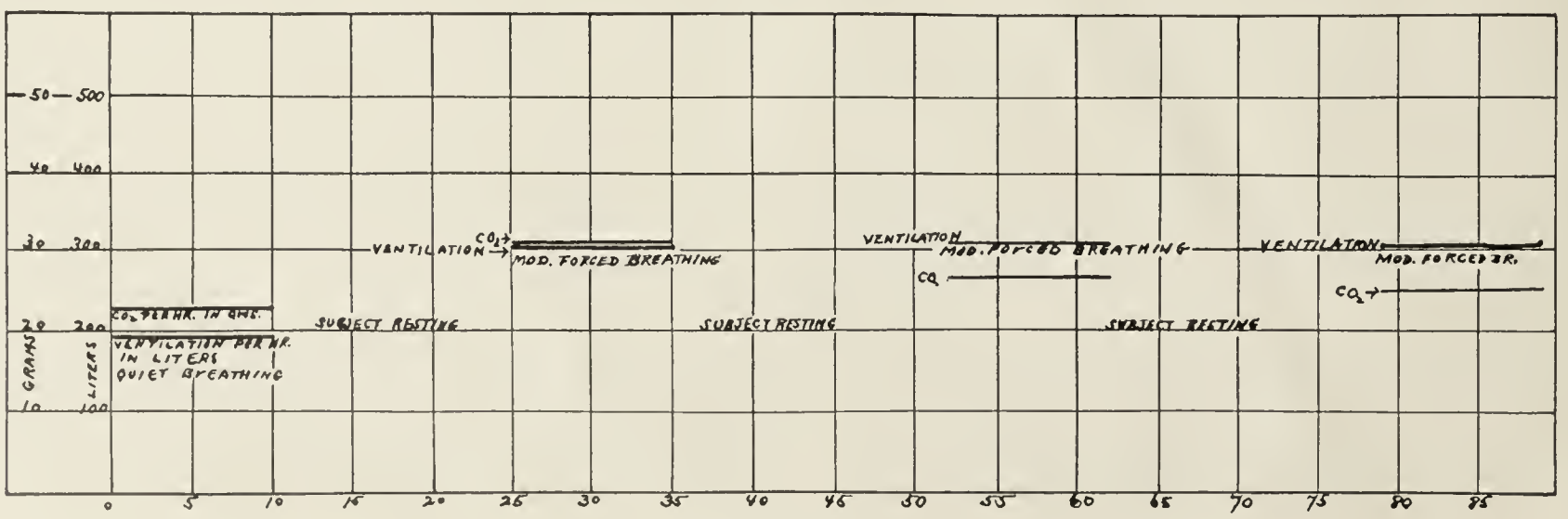


Fig. 5.

There develops from this study one condition under which the CO_2 output may be raised above the limits of normal and in which a "check" of a little over 5% may be obtained. This is Experiment 4, in which the ventilation rate was moderately increased and maintained at a constant level. We have no way of reproducing exactly the reaction of a patient to mouth breathing, and we can only state that a patient might duplicate Experiment 5 in the course of a metabolism experiment, provided he maintains a ventilation rate at a constant level regardless of his subjective sensations.

In order to determine whether excessively high CO_2 figures are actually obtained upon patients other than in hyperthyroidism or some similar condition which elevates the metabolism, we have analyzed 200 cases studied by a technician. These were routine tests done upon dispensary and hospital patients. They were all done upon patients in a strictly basal condition, so far as dispensary patients are ever "basal." They were not done with thought of publication and are consecutive cases. Miss Cross was instructed to consider no test satisfactory unless the CO_2 output for the two periods checked within 10%. If the results indicated that the true results were somewhere about the border-line zone, a closer check was obtained. Such generous limits were allowed, since it has been found that CO_2 figures for consecutive periods seldom check quite so closely as do O_2 figures, and we feel that the results justify the adoption of no more stringent rules for a check than the ones given.

TABLE I.

BASAL METABOLISM	RESTING PULSE								CLINICAL DIAGNOSIS					
	50-60	60-70	70-80	80-90	90-100	100-110	110-120	120+	0	0-1	1	2	3	
+100%														
+90%								1					1	
+80%								1					1	
+70%							1						1	
+60%					2	2							4	
+50%					1	1	1	1					4	
+40%				2	1	1	1	1				1	5	
+30%			2[6]	1	1	2	2				1[F]		7[6]	
+20%			1[A]	1[6]	1		3		1[A]			1[6]	4[7]	
+15%			4[E]	5[D]	2	3	2		2[D]	3	4	3	4	
+10%														
AVERAGE		5	15	13	11	2			31	6	5	3	1[J]	
NORMAL -10%	2	8	23	21[B]	6	2	1	-	50	4	3	1	2[G]	
-15%	2	7	7	5	1				18	3	1			
-20%	2	5	9	2					17	1				
-30%	1	2	1	2					6					
-40%					1					1				
-50%														
			TOTAL - 200							SAME 200 TESTS				

200 Consecutive tests by CO_2 method. (See key.)

TOTAL—200 Cases
Key to Above:

A—Cardio-Nephritic. Secondary anæmia.
B—Results in same case before and after thyroidectomy. No change in pulse or B. M. after operation.
C—Pituitary Disease.
D—Pulse 82-96. No definite thyroid signs. Osteosclerosis.
E—Syphilis. Slight goitre.
F—C-N-S. Lues. T. to 99.6°.
G—Pulse normal. Protrusio. Von Graefe. Rosenbach.
H—Toxic Adenoma.
I—Hyperthyroidism.
J—Adenoma, slight symptoms. Normal pulse. B.M. +7%.

TOTAL—200 Cases (same).
Key to Clinical Diagnoses.

O=Those cases in which no diagnosis of hyperthyroidism was made clinically.
This group includes all cases of suspected hypothyroidism.
O—1=Cases which gave some questionable indication of hyperthyroidism.
1=Cases in which hyperthyroidism was suspected.
2=Cases with diagnosis of probable hyperthyroidism.
3=Cases in which a definite diagnosis of hyperthyroidism was made.

If patients should produce by superventilation falsely high CO_2 figures that "check" for consecutive periods, it would be possible to find some indication of such results in a comparison of the CO_2 output with the clinical condition of the patient in a large number of cases. Such a check upon the metabolic rate determinations is routine at the Mayo Clinic, I understand, and it is justified by common sense. In this series of 200 cases, however, there was no reference to the clinical condition of the patient when the test was made, and Miss Cross was not trained accurately to determine such clinical condition. It is fair to say, therefore, that the tests were done and reported without prejudice from the clinical aspect of the case, and the comparisons were not made until the end of the series.

The first comparison is that of the metabolic rate and resting pulse determinations for each case.

A general correlation between the metabolic rate and pulse rate is obvious. The line separating the pulse rates of 80-90 per min. from those of 90-100 was doubled to bring out the observation made by Sturgis and Tompkins [8] that most patients with hyperthyroidism have resting pulse rates of 90 or more per minute, while most patients with normal basal metabolism have pulse rates less than 90. In our series of 200 cases there are 19 patients in whom a pulse rate of 90-100 per minute was associated with a metabolic rate of less than 10% plus. There are only 5 cases in which the resting pulse was over 100 in which the metabolic rate was found below 10% plus. There are 3 cases ("6"—a suspected hyperthyroidism; "7"—a toxic adenoma case; and "A"—a clear case of hyperthyroidism clinically) in which a basal metabolism over 15% plus was associated with a pulse of 70-80. One patient with pituitary disease ("3") had an increased metabolic rate and a pulse of 80-90. The cases of 3 patients with a pulse of 80-90 and a metabolic rate of more than 20% plus were clear instances of hyperthyroidism clinically.

[8] Sturgis, C. C. and Tompkins, E. H.: Correlation of Basal Metabolism and Pulse Rate in Patients with Hyperthyroidism. Arch. Int. Med., 1920, XXVI, 467.

The second comparison is of especial aid in judging the value of the estimation of the CO_2 output as a clinical index to hyperthyroidism. There was only 1 case out of the 200 in which the diagnosis of hyperthyroidism was not suspected and in which a metabolic rate of more than 15% plus was obtained. This was a man with cardio-renal disease, in which the basal metabolism is frequently elevated, according to Peabody, Meyer and DuBois.[9] There is another case, "6" (see key), in which hyperthyroidism was suspected and in which the basal metabolism was between 20 and 30% plus. After studying the history and the opinions of the clinicians, I feel that this patient may be classed as one with hyperthyroidism. A third patient with a metabolic rate over 15% plus had pituitary disease, a possible cause of increased metabolism.[10] A fourth patient with a diagnosis of probable hyperthyroidism had a metabolic rate over 30% plus.

There is, then, an obvious cause to explain the elevation of the CO_2 output in each of the 4 cases out of 200 in which a clear cut diagnosis of hyperthyroidism was not made and in which the metabolic rate was elevated more than 15%. We have therefore been unable to find any instance in which we feel that an increased CO_2 elimination was produced "artificially"—either by restlessness or by superventilation of the lungs, in this series of 200 determinations.

We have naturally asked ourselves why, since it is possible to increase the CO_2 elimination so readily by superventilation, we do not get cases in which normal people give abnormally high CO_2 figures. We believe that the sharp increase in CO_2 output that results from superventilation develops into a real aid rather than otherwise in the practical application of the test. I do not recall a case in which the patient was obviously superventilating the lungs in which a satisfactory check was obtained with the second period. A review of the various experiments in this report shows that the bulk of dissociable CO_2 is washed out promptly by most degrees of forced breathing, leaving comparatively little to be washed out in the following period. Such a test shows so great a discrepancy in the CO_2 output of the two periods that it is discarded. Tests of this kind have not been included in the series reported here. We have not kept a record of the number of such rejected tests, but there were several of them in the course of these determinations.

Another factor that also undoubtedly interferes with falsely high CO_2 elimination is the physiologic tendency toward retention that begins to assert itself whenever there occurs an excessive liberation of CO_2.

[9] Peabody, F. W., Meyer, A. L., DuBois, E. F.: The Basal Metabolism of Patients with Cardiac and Renal Disease. Arch. Int. Med., 1916, XVII, 980.

[10] Means, J. H.: Studies of the Basal Metabolism in Obesity and Pituitary Disease. Jour. Med. Res., 1915, XXVII, 121.

In this series of 200 observations, then, there are only 3 cases in which hyperthyroidism was not suspected (it was not suspected in 125 instances out of 200) in which the basal metabolism was in excess of 10% plus; these were one case of cardio-renal disease, one of osteosclerosis with tachycardia, and one of syphilis with goitre. In these instances the degree of super-normality was slight in each.

We are able to compare with this group a recent report of 668 observations upon all types of patients by Means and Burgess,[11] all of which were made with the closed system oxygen-rich method. During the first three years over which these data were being collected both oxygen consumption and carbon-dioxide elimination were measured and the heat production was calculated therefrom. During the last five years only the oxygen was calculated and a R.Q. of 0.82 was assumed. The majority of these observations of Means are based upon oxygen figures alone; the rest depend chiefly upon the O_2 figures, since the caloric equivalent of oxygen varies not more than 3.7% whatever the CO_2 figures may have been.

Out of this series of 668 cases studied by Means and Burgess, omitting the obvious cases of hyperthyroidism and hypothyroidism, may be found 378 cases (57%) in which hyperthyroidism was not suspected. In our series of 200 cases it was not suspected in 62%. Among their group of 378 cases may be found 97, or about 26%, in which supernormal metabolic rates were found. Among the 125 cases of our series that fall into the same category only 3, or 2.4%, show supernormal metabolic rates.

If we eliminate the groupings "non-toxic goitre," "suspected hypothyroidism," "endocrine disturbances," and "blood diseases" from the series of Means and Burgess, there remains a group of 99 observations labeled "miscellaneous, non-endocrine," which form the residue of the cases. Even in this group 20.2% showed supernormal basal metabolism. About 7% of these are explained in various ways.

It is quite obvious, therfore, that there exists a very much more marked liability toward supernormal oxygen consumption among patients who are not suffering from hyperthyroidism than there is a liability toward increased CO_2 elimination among similar patients. One might suppose that our CO_2 standards of normal are too high. The scale of normal figures by which we have been guided were proposed by me.[12] They are roughly 1% lower than those obtained from an analysis of a large number of normal subjects by Benedict and others.[13] They are 1%

[11] Means, J. H. and Burgess, H. W.: The Basal Metabolism in Non-Toxic Goitre and in Borderline Thyroid Cases. Arch. Int Med., 1922, XXX, 507.

[12] King, J. T. Jr.: Determination of the Basal Metabolism from the Carbon-Dioxide Elimination. Johns Hopkins Hosp. Bull., 1921, XXXII, 367.

[13] See 12.

higher than the figures constructed by me on the basis of results from 17 normal men obtained by the use of the CO_2 collecting apparatus used in these 200 observations.

In view of the results published in a previous paper,[14] which showed the excessively high O_2 absorption during the early part of DuBois's experiments with hyperthyroid patients and in view of the comparatively poor correlation between O_2 absorption and heat production reported previously,[12,14] it seems to us fair to conclude that there is a distinct danger in the use of an oxygen absorption apparatus in the direction of falsely high figures. This is not the case with the measurement of CO_2 elimination. Perhaps half of our observations were upon dispensary patients, who would give false supernormal figures if there were any such tendency.

Taking the 200 cases in this series together we find a total of 107 cases below the normal average and 93 above the average. Considering plus 10% as roughly the limits of normal we find 47 supernormal observations and 47 subnormal observations. There is a sharp difference here between our results and those of Means and Burgess, who found a total of 50.1% supernormal and of 6.3% subnormal observations. Leaving out cases of clear clinical hyperthyroidism and hypothyroidism, these authors found supernormal metabolic rates in 30.2% and subnormal rates in 9.3%.

We have considered the possibility that our results might be falsely low through some technical difficulty. As stated above, the normal figures of CO_2 elimination obtained by the apparatus used in all these observations were 1% lower than the scale of normal figures that have been used routinely. If our scale should be at fault, therefore, there is reason to feel that it is only 1% too high, which might throw all our results 1% below the reported figures. Again, if there is a technical difficulty causing falsely low results, it should appear in the frankly hyperthyroid group of cases (Col. 3). Case No. 2 of this group, shown just below the line of normal was a patient with a large irregular goitre, slight tremor, pulse 80-90. The pre-operation metabolic rate was about minus 2%. After operation the pulse rate, metabolic rate and clinical condition were unchanged. I do not feel that this case really belongs in Col. 3, but I have put it there because of the written hospital diagnosis. Case "B" shown just above the normal level had a small thyroid adenoma and very mild symptoms, with a basal metabolism plus 7%. In every other clear case of hyperthyroidism, of which there are 31 in all, the basal metabolism was supernormal. We have, therefore, been unable to establish any tendency toward falsely low results in this group, in which, in fact, the clinical diagnosis offers us the most valuable check upon our results. The efficacy of the soda lime in absorbing CO_2 may be tested by passing the expired air through a saturated solution of barium hydroxide. A cloudy precipitate will indicate that CO_2 is getting through the soda lime without being attached. If Wilson soda lime is used, it is safe to allow the 2. liter jar to absorb 75 grams (net) before filling with fresh soda lime.

There is also a series of cases reported by Boothby and Sandiford,[15] studied by the Tissot method. Leaving out all cases with clinical hyperthyroidism they found that 15.8% of the residue, including clinically hypothyroid cases, had metabolic rates of less than minus 10%. Of this group 8.9% had subnormal metabolic rates if the hypothyroid cases are left out.

We may now tabulate the results of three series of cases, to show the tendency toward increased metabolic rates among cases that are not clinically hyperthyroid, as determined by 3 different methods. Cases of malignant disease of the thyroid and of thyroiditis are excluded, also the group of colloid goitres of Boothby and Sandiford, which contains some cases of adenoma.

Observer	Method	No. of cases not suspected of hyperthyroidism	% of cases not clinically hyperthyroid with metabolism above plus 10%
Boothby & Sandiford	Tissot-Gasometer	2,674	12.7
Means & Burgess	Benedict	378	25.7
The Author	CO-2 Elimination	125—157	2.4—7.0

In the third series (the author's) the first figure (125) represents all cases which had no clinical evidence of hyperthyroidism; of these 2.4% had metabolic rates in excess of plus 10%. If we include cases in which hyperthyroidism was considered from the clinical standpoint a possibility, though not a probability, the number is increased to 157; of these 7% had supernormal metabolic rates. Some of the latter cases were probably true cases of hyperthyroidism. Dr. E. W. Bridgman and the author have used the CO_2 method in perhaps 800 observations upon private patients and, though no analysis of these has been made, I believe the 200 consecutive cases in this report are a representative group.

Through the kindness of Drs. Hannon and Lyman we are able to make comparisons in a few cases of results obtained by different methods upon the same patients.

[14] King, J. T. Jr.: See Johns Hopkins Hosp. Bull., 1923, XXXIV, p. 304.

[15] Boothby, W. M. and Sandiford, I.: Summary of the Basal Metabolism Data on 8,614 Subjects with Especial Reference to the Normal Standards, etc. Jour. Biol. Chem., 1922, LIV, p. 783.

Comparison of results from different types of apparatus.

Name and Diagnosis	Metabolor	Tissot	CO_2
M. E. Normal	—5 (1/21/23)	—2.5 (1/21/23)	
F. S. Tb. Kidney	—10 (1/18/23)	—9 (1/18/23)	
E. W. R. C. N. S. Lues	—9 (1/20/23)	—4 (1/20/23)	
C. B. Hyperthyroid (Post.-op.)	+26 (1/8/23)	+31 (1/8/23)	+29 (1/2/23)
J. J. Hyperthyroid	+42 (1/5/23)	+47 (1/4/23)	+50
W. A. Diabetic	+2 (11/9/22)	—4 (11/10/22)	
G. H. Diabetic	—3 (10/11/22)	—1 (11/19/22)	
I. P. Diabetic	—3 (11/15/22)	—3 (12/20/22)	
R. W. Diabetic	—21 (11/10/22)	—17 (12/27/22)	
F. H. Leukæmia	+41 (12/2/22)		+44.6 (12/29/22)
C. H. Hypothyroid	—8 (12/1/22)		—10.5 (12/21/22)
R. J. Hysterical hyperpnœa	+22 & +31 (11/21/22) (poor record)		
	+19 (12/9/22)		+13.2 (12/8/22)
T. K. Hyperthyroid	+29 (12/5/22)		+35 (12/14/22)
			+29.9 (12/29/22)
I. K. Hyperthyroid (?)	+37 (12/11/22)		
			+5.7 (p. 72-80) (3/3/23)
	+21 (4/3/23)		+18.7 (p. 82-90) (4/3/23)

From these direct comparisons we are unable to see any evidence of washing out of CO_2. Where the difference in the results by various methods is marked (as in R. J., hysterical hyperpnœa and I. K., case of questionable hyperthyroidism) the CO_2 figures are lower.

Discussion

The experiments with superventilation of the lungs have been discussed rather fully.

As to the comparison of the metabolic rate with the clinical condition of the patient, in these 200 cases we might repeat that the correlation seems to us ideal in the cases in which the metabolic rate is above the line of normal. We are more familiar with the clinical aspects of such cases than we are with the clinical evidences of a lowered metabolism. Inasmuch as this report has to do with the possible effect of superventilation upon the CO_2 output, we have not gone into the cases of lowered metabolism, of which there are exactly as many as there are of increased metabolism. There are more cases below the line than there are in the report of Means and Burgess. We believe the number of such cases is due to the type of case studied, a large number of which represented private patients suffering from menopause symptoms, complicated with hypothyroidism, with a rather large incidence of thyroidectomy cases, oöphorectomy cases, colloid goitres and myxœdema. Many of them were treated with success, and repeated determinations of the basal metabolism while under treatment verified the technical findings in all the cases that were so followed.

The comparison of the metabolism determinations by the three methods seems to us highly suggestive. The incidence of supernormal metabolic readings among patients not clinically hyperthyroid by the Benedict method is twice as high as it is by the "open" gasometer method of the Mayo Clinic. The number of such cases studied is large enough in each group to show "how the wind blows." In view of the reports of anoxemia in certain diseases and the demonstration that patients and even normal subjects [16] may take up an increased amount of oxygen into the blood provided it be presented in a concentrated form in the inspired air, it seems to us that the simplest and most likely explanation of the discrepancy between the findings in these two series would be the assumption that the large number of patients in Means and Burgess's series who absorbed excessive amounts of oxygen did so because the oxygen was presented to them in a concentrated form at a time when there existed a certain grade of anoxemia. I do not see how we can escape the danger of the patient's absorbing more oxygen than is being used in his immediate metabolism if it be present almost in a pure state in the inspired air, particularly if the condition be complicated by cardiac decompensation.

Summary

1. Superventilation of the lungs elevates the carbon-dioxide elimination for ten and fifteen-minute periods. If the superventilation is brisk most of the dissociable carbon-dioxide is eliminated in the first period; the CO_2 elimination for the second period may be very little above normal.

2. If superventilation be moderate, the CO_2 elimination may check within a little over 5% for two consecutive ten-minute periods, provided the superventilation be maintained regardless of the urge toward apnœa.

3. In a series of 200 consecutive determinations of the basal metabolism from the carbon-dioxide elimination

[16] Barach, A. L. and Woodwell, M. N.: Studies in Oxygen Therapy. I and II. Arch. Int. Med., 1921, XXVIII, 367, 394.

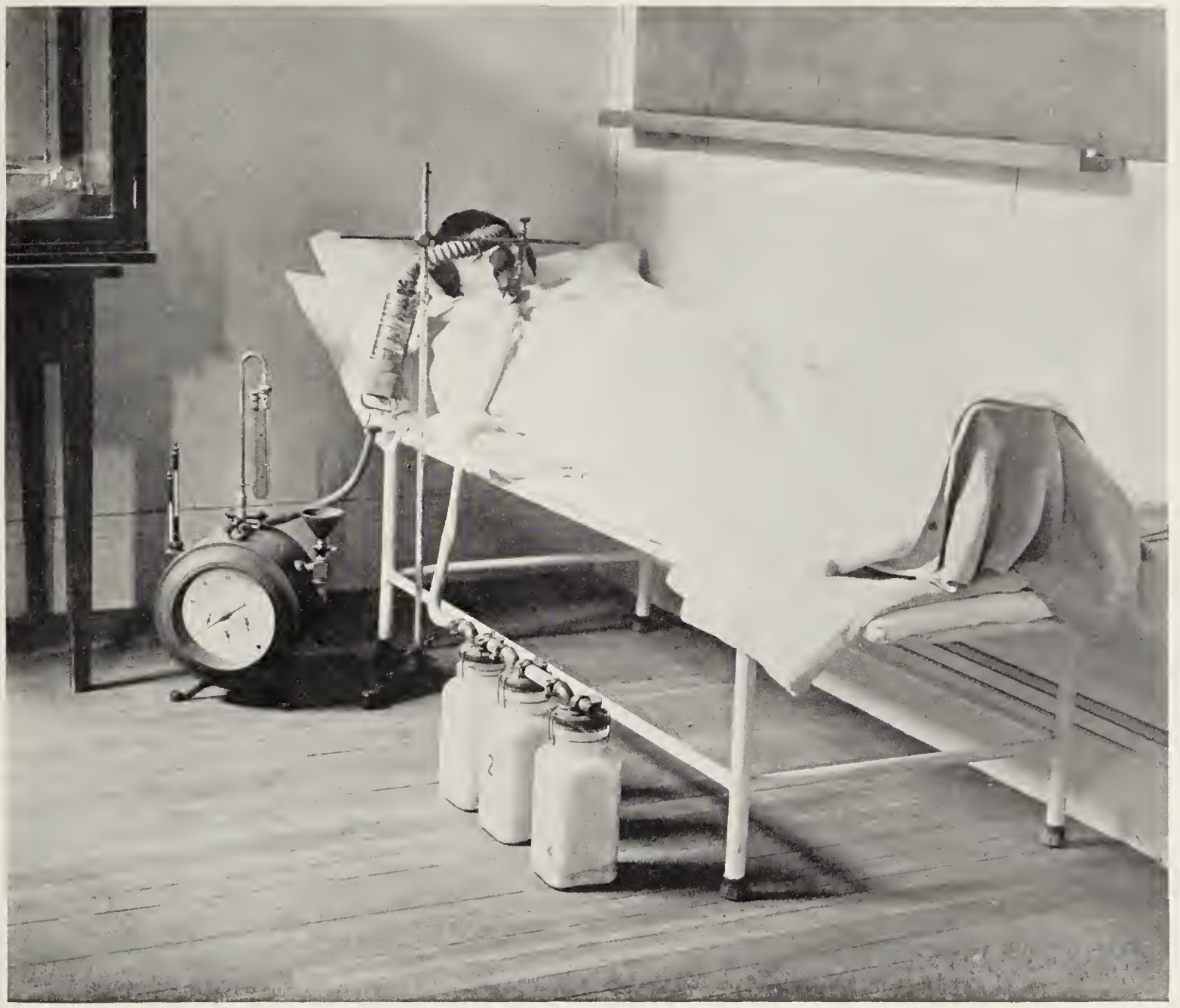


Fig. 6.—Apparatus with experimental meter attached.

there was no elevation of the carbon-dioxide elimination that was not supported by clinical data.

4. Among patients in our series not suspected of being hyperthyroid there were supernormal (above plus 10%) metabolic rates in 2.4%. This percentage cannot be raised above 7% by including all borderline cases of hyperthyroidism.

5. A comparison of the number of supernormal metabolic rate determinations among patients not suspected of hyperthyroidism as shown by three methods is:

Observers	Method	Percentage
Boothby and Sandiford	Tissot Gasometer (Open System)	12.7
Means and Burgess	Benedict (Closed System)	25.7
The author	CO_2 Elimination (Open System)	2.4—7

Conclusions

1. It is experimentally possible to increase the carbon-dioxide elimination through the lungs to nearly the same level for two consecutive ten-minute periods.

2. In the determination of the basal metabolism by the CO_2 elimination method two periods of observations should always be used. If this is done, it becomes simple to recognize the effect of superventilation by the sharp drop in CO_2 output in the second period. We have been unable to find any indication that falsely high CO_2 figures are obtained by this method through superventilation. If superventilation occurs, it is best to repeat the test on another day because of the subsequent retention of carbon-dioxide.

3. If there is hesitancy in using this method for fear of the effects of superventilation, a commercial gas meter may be placed between the patient and the source of fresh air and an approximate estimation of the ventilation rate obtained therefrom.

4. If the basal metabolism is determined by a closed oxygen-rich system, such as the Benedict, twice as many supernormal results may be expected among patients not clinically hyperthyroid as would be obtained by the gasometer method. Among the same group of cases more than twice as many supernormal results may be expected by the Tissot-gasometer method as by the measurement of CO_2 elimination.

5. It seems likely that excessively high results may be obtained by the closed system oxygen method because of the compensatory absorption of oxygen by patients and even by normal subjects.

A SUBSTITUTE FOR ACID HEMATIN AS THE STANDARD IN SAHLI'S HEMOGLOBINOMETER

By Lloyd D. Felton

(From the Pathological Laboratory, Johns Hopkins Medical School)

Among the clinical methods for the estimation of hemoglobin, the Sahli modification of the Gowers method is the most widely employed and gives, perhaps, as dependable results as any of them. The estimation is rapid; the apparatus is simple and convenient; the acid hematin color as easily matched as the red color of the Dare or Fleischl-Miescher apparatus; and the results are reproducible to within 2 to 5 per cent, this degree of error being much less than the percentage of change from the normal amount of hemoglobin to that which may indicate a pathological state. In the hands of the observer who possesses an accurate color sense, which is necessary for all the commonly used methods for clinical purposes, a sufficiently dependable estimation of hemoglobin can be made with the Sahli hemoglobinometer. The disadvantages of this instrument, however, are the difficulty experienced in preparation of the color standard (acid hematin) and the uncertainty of the standard color, due to a more or less rapid fading. In consequence of these disadvantages, the color of the standard is relative and has resulted in empirical colors: some clinics prefer to use the original idea of Sahli, that the average normal reading be 90 per cent; others, his more recent standardization, that the highest possible normal be 100 per cent; and still others standardize their Sahli by means of the Fleischl-Miescher apparatus, using an absolute standard of 14.3 grams of hemoglobin to one liter. This does not represent a definite hemoglobin percentage, but a varying value in the different laboratories of 90, 95 or 100 per cent. At best, the standard is of uncertain color strength and the possible difference in the estimated percentage of hemoglobin in the various clinics by the Sahli method may vary from 20 to 30 per cent. This lack of uniformity and the difficulty experienced in preparing and maintaining a constant standard suggested the need of a chemical substitute for acid hematin, which possessed permanency of color.

While the problem was still in mind, it was observed that black tea steeped in a soft glass beaker became very dark on standing, and seemed to have the color of a concentrated acid hematin solution. On trial, the unfiltered tea infusion was found to be such a perfect match for the acid hematin standard that four men in the laboratory were unable to distinguish between the hematin

solution and the tea infusion. The tea was diluted to the color tint of the hematin standard and four Sahli tubes filled and sealed. One of the tubes was placed so that it was in direct sunlight about 50 per cent of the day; another remained in ordinary laboratory light; a third was placed on alternate weeks in the dark and in the laboratory light; and a fourth was kept in the dark with the Sahli acid hematin standard. The tea infusion was read from month to month and was found to be stable in color. The infusion itself exposed to sunlight for about two years became approximately two per cent darker, while no change was noted with the other tubes during this length of time. Subsequent work showed that it was mere chance that this lot of tea infusion had undergone complete oxidation, for other attempts to make a constant color concentration with an infusion of the same tea were met by failure, until a definite amount of alkali was added to the infusion. This fact drew our attention to the well known brown oxidation products of the tannins, gallic acid and pyrogallic acid in alkaline solution.

It was found possible to make a color standard very nearly matching the acid hematin with any of the three chemicals mentioned above. The operation with the tannins was rather tedious, requiring a temperature of about 90°C. for a period of two or three weeks. With gallic acid, oxidation progressed much more easily than with the tannins, but some difficulty was experienced in obtaining the correct color. This difficulty was perhaps due to the difference in H-ion concentration, which was found later to be the fact with the oxidation product of pyrogallic acid. The oxidation product of pyrogallic acid in alkaline solution was the easiest to handle and gave nearly reproducible preparations. The complexity of the chemical compounds arising from the oxidation of these three chemicals in alkaline solution makes it impossible to specify the chemical which possesses the characteristics whose solution gives a color so nearly like acid hematin. Indeed, it is disappointing that the chemical which met the requirements as a substitute for acid hematin fell in the group of organic chemicals the exact composition of which is not known.

The ideal substance for use as a color standard would be one of definite chemical constitution, a weighed portion of which could be dissolved to give the required color intensity. This, however, being impossible for the present, attempts were made to prepare the alkaline oxidation product of pyrogallic acid in dry form. It was found that this oxidation product was insoluble in 75 per cent ethyl alcohol. This fact suggested the possibility of oxidizing the pyrogallic acid in 75 per cent alcohol, the oxidized substance being thus precipitated as fast as formed. The method was tried and gave satisfactory results; the oxidized substance being thrown down in the form of a black, sticky mass which could be washed, dried and subsequently ground into a fine powder. The substance so obtained is freely soluble in water; dissolved in a solution of the proper H-ion concentration, it gives a color indistinguishable from acid hematin when used in the Sahli instrument.

The technique used in preparing the powder is as follows: a ten per cent solution of pyrogallic acid is made in 75 per cent ethyl alcohol and enough 40 per cent sodium hydroxide to make a 10 per cent solution of the alkali in the 75 per cent alcohol. The resultant mixture is oxidized by bubbling air through it until no more precipitate is formed, overnight being sufficient. The supernatant fluid is decanted and the tarry mass dissolved in water. It is again precipitated by 75 per cent alcohol. The precipitate is washed in this way until it becomes neutral in reaction, or at least is not strongly alkaline. After washing with 95 per cent alcohol, the substance is spread out in a thin layer until no odor of alcohol is detected. After drying in a sulphuric acid dessicator, the product is ground into a fine powder.

On dissolving the powder preparatory to standardization, it was observed that a yellow tint was present in the solution which did not exist in the liquid preparation made previously. The substance proved to be a very rough indicator of H-ion concentration. On the acid side there appeared a reddish shade to the brown color, and on the alkaline side a yellowish one. It was thus obvious that the previous difficulties in getting the exact color match, especially with the tea infusion, could be explained in the different H-ion concentration of the tea infusion; at one time the preparation having a yellowish cast and at another a reddish one. The H-ion concentration which gives the best color match is neutrality, that is, a pH of 7. However, when the powder is dissolved in Sørensen's phosphate buffer mixture at a pH of 7, a slight precipitate is gradually formed which causes change in the color. This does not occur if a buffer of pH 6 is used as a solvent and the color match is nearly as exact as when the solution has a pH of 7.

The criteria for a color standard suitable for use in the Sahli hemoglobinometer which is superior to acid hematin are that the color must be the same as that of acid hematin and maintain a constant color tint. The first of the conditions has been met. Different observers have failed time and again to distinguish between the acid hematin and the dye, provided of course the two were viewed through ground glass. How close the color match really is is shown in Table I. In this experiment a

TABLE I.

Comparison of acid hematin and the dye

	DILUTION IN PERCENTAGE							
	100	90	80	70	60	50	40	30	20
	DUBOSQUE READINGS								
Acid hematin	1.3	1.6	1.8	2.21	2.7	3.4	4.2	7.2	11.4
Dye	1.3	1.58	1.77	2.2	2.86	3.6	4.5	7.8	12.6

Average of 5 observations

solution of acid hematin made from fresh human blood was adjusted in color by means of the Dubosque colorimeter to match a given concentration of the dye. Different percentages of these stock solutions were made and compared with the original stock of acid hematin. The match is very close down to 60 per cent where the color strength of the dye solution begins to decrease with greater rapidity than acid hematin. In this particular experiment, in the solution of the dye representing 60 per cent and below, a yellowish cast of the color became prominent, suggesting that the H-ion concentration was at fault. Although the color match in high concentration can be relied on, it is possible that a more accurate adjustment of the pH value of the solution may insure a better match of color in the lower dilutions.

Permanency of color is the second criterion. To test this characteristic, two types of experiment were carried out, namely, first, a standardized solution subjected to different degrees of light over a long period of time, in parallel with acid hematin, and, second, influence of varying amounts of heat on this standard solution. In a preceding paragraph, it has been mentioned that the original tea standard maintained a permanency of color for over two years, the tube exposed to bright light becoming slightly darker. A more systematic experiment is represented in Table II, where acid hematin is exposed together with the dye for a period of 18 months. The method of procedure in testing the standards was, first, to compare them with the hemoglobin percentage of an individual who was found to possess a very constant percentage of hemoglobin. After three tests had been made, the dye kept in the dark was used as standard and only at the end of the 18-month period was a careful estimate made. Although there exists some uncertainty as to the final value of the four standards which were compared, there is no doubt but that the acid hematin is inferior to the dye in regard to permanency under the conditions of this experiment. The second test for permanency, subjection to different amounts of heat, confirms this experiment. Five cubic centimeters of a standard dye solution, 100 per cent as compared with acid hematin, were sealed in a 20 c.c. test-tube and autoclaved at 15 pounds pressure for 15 minutes. The color of the dye after the standard had cooled was 10 per cent higher than before autoclaving. This experiment was repeated with four tubes instead of one, a tube being taken from the autoclave each 15 minutes for 4 intervals; thus, the tubes were subjected to approximately the same temperature for varying lengths of time—15 minutes, 30 minutes, 45 minutes and 60 minutes, respectively. The four tubes of standard were indistinguishable in color intensity after heating and were about 10 per cent higher than the unheated solution. The explanation of this change in color dye solution subjected to heat is not certain. There is a possibility that it is a pH phenomenon or perhaps the result of more complete oxidation of the dye caused by the high temperature. However, the explanation of the change in color intensity is not within the scope of this work, inasmuch as we wish only to ascertain from these few experiments whether or not we have found a dye which may be used as a suitable substitute for acid hematin in the determination of the percentage of hemoglobin in the Sahli apparatus. From the experiments given in this report, and from the opinion of a great many laboratory men who are using the dye standard which I had prepared, I feel justified in advising the use of this alkali oxidation product of pyrogallic acid as a color standard in substitution for acid hematin in the Sahli hemoglobinometer. From my experience, I feel certain that a uniform product may be prepared, a weighed portion of which will give a precise color intensity; so that a certain percentage of the dye will represent an accurate concentration of hemoglobin.

TABLE II.

Comparison of acid hematin and dye in permanency

STANDARD	READINGS AT 2 WEEK INTERVALS								
	2	4	6	8	10	12	14	16	18
Acid hematin 90% 50% in light	82	79	61	58	52	47	47	47	47
Dye 90% 50% in light	90	90	90	90	90	90	92	92	*92
Acid hematin 90% in dark	90	90	90	90	88	88	88	88	85
Dye 90% in dark	90	90	90	90	90	90	90	90	90

* This tube was kept for over two years as a standard and suffered no appreciable change.

NOTES ON NEW BOOKS

The Difficulties and Emergencies of Obstetric Practice. By Comyns Berkeley, M. D. and Victor Bonney, M. D., London, England. Cloth $11.00. *(Philadelphia, P. Blakiston's Son and Co., 1921.)*

To enumerate for the "family physician" the abnormalities of any specialty without due regard to the normal is to engender in the average doctor a lack of respect and utter disregard for that specialty, and to give him a false self-confidence. In Obstetrics, probably more than in any other branch of medicine, the determination that any condition is abnormal, presents a difficulty, or constitutes an emergency, is so intimately bound with the accurate recognition of the normal that it seems unwise to attempt, as is done in this book, to divorce the two. In fact, since many obstetrical mishaps arise as a direct result of the ignorance of the attending physician, one must know the normal in order properly to treat the abnormal.

For these reasons, therefore, we have never seen the need for this or any similar book, and can only concede its value in so far as it may impress the general practitioner of his unfitness properly to care for the pregnant, parturient, and puerperal

woman, and cause him to spread the propaganda for reform in midwifery.

Many additions have made the third edition of this book a veritable *Encyclopaedia Britannica* of obstetrical pitfalls and accidents.

The subject matter is handled in only mediocre fashion. It is rather surprising to find no mention of transfusion, (although saline infusion is described as an operation). If this procedure is not approved of, it should be condemned. To ignore transfusion entirely creates the idea that its beneficial effects in treating shock and its life-saving results in severe hemorrhage are unknown. Eclampsia is considered under "The Disorders of the Urinary Tract," and is dealt with so briefly that one gains the impression that it is thought to be a minor emergency rather than one of paramount obstetrical importance. Strangely enough, the search for and the recognition of the early signs of this toxaemia, and the treatment of the pre-eclamptic condition, are not considered. Syphilis may not be as prevalent in Great Britain as in our country, but, even so, as it is recognized to be the greatest single causative agent in the production of still-births after the period of viability, it could with profit to the average doctor be given more prominence. We deplore, in these days of excellent results with properly employed Caesarean Section, the advocacy of the induction of premature labor for contracted pelves. The general use of this procedure will result in its employment unnecessarily many times and cause the disasters incident to infection and prematurity. In a book written for the family doctor it seems unwise to devote almost fifty pages to the various types of Caesarean Section and less than twenty to the use of the forceps. The general practitioner uses forceps more often than he does a laparotomy, but too often he is more competent to perform the latter than the former operation. Certainly, he is more often cursed with severe perineal lacerations than his trained colleague, yet for his benefit but a few words in one sentence offer instruction in the suturing of a torn sphincter ani muscle, and the other details of perineal repair are superficially considered. The chapter on "Diseases and Injuries of the New Born Child" is very satisfactory, although congenital atelectasis is omitted. The chapter on "Feeding of Infants" is excellent and will fill a definite need.

The book is attractively printed and, although heavy to handle, is easily read. By discarding some of the superfluous illustrations, and by a more compact arrangement of the text, paper and printing could be conserved and the book made less expensive, for its present cost is excessive in comparison to its value.

J. G. M., Jr.

Physics and Chemistry for Nurses. By A. R. Bliss and A. H. Olive. *(J. B. Lippincott Co., Philadelphia, 1923.)*

This little book presents in a concise and very complete way the fundamental principles of physics and chemistry that are of most interest and importance to a nurse.

Although physics is not taught in the Johns Hopkins Training School, the first portion of the book has proved valuable to us for reference work. The part devoted to chemistry has been most useful, but if it were less technical it would be better suited to our purposes.

L. O. C.

Practical Nursing. By Anna C. Maxwell, R. N., M. A., and Amy E. Pope, R. N. 4th Edition. $2.50. *(New York and London: G. P. Putnam's Sons, 1923.)*

The fourth edition of Practical Nursing by Maxwell and Pope includes not only the essentials necessary in the care of the furnishing and equipment of the hospital, the nursing points to be observed in the commoner medical diseases and surgical conditions, but explains the actual nursing procedures in minute detail, placing stress upon the care and comfort of the patient. The most recent treatments and methods are included in this edition.

The long experience of both authors as administrators and teachers has given them exceptional opportunities to understand the needs of the nurse, and the book is valuable not only as a text-book for the student nurse but also as a guide to those teaching nursing subjects.

H. K. F.

Encephalitis Lethargica. By A. C. Parsons, A. S. MacNalty, and J. R. Perdrau. Reports on Public Health and Medical Subjects. No. 11. *(London, Ministry of Health, 1922.)*

Collective reviews of experiences with epidemic encephalitis have, since 1917, been appearing from time to time in each of the several countries in which the disease has been prevalent. In England, two such reviews have been published by the Ministry of Health, the first in 1918 by MacNalty and James, and the second in 1922, now before us for comment, by Parsons, MacNalty and Perdrau.

In the first report, the opinion, based upon clinical, pathological and epidemiological inquiries, was expressed that lethargic encephalitis is not to be included in the Heine-Medin group of diseases (to which acute poliomyelitis belongs), but is a disease *sui generis*, with characteristic clinical features as described in Vienna by von Economo in 1917. As a result of the studies embodied in the report of 1918, it was recommended by Sir Arthur Newsholme that the disease be made compulsorily notifiable and this measure was carried out, beginning with January 1st, 1919.

In the second report, now before us, studies of the disease (1250 cases) made during 1919 and 1920 are summarized, facts bearing upon epidemiology and etiology are discussed, the clinical pictures (both typical and atypical) met with are fully described, and certain observations upon the pathological histology in man and the experimental production of the disease in animals are recorded.

Among the features emphasized in this last report may be mentioned:—(1) the tendency to prevalence in the winter months; (2) the distinction between the disease and both botulism and acute poliomyelitis; (3) the failure to find any evidence of any clear and direct epidemiological or clinical relationship with influenza; (4) the frequent confusion of the disease in the young with tuberculous meningitis and in the older with cerebral tumour or cerebral hemorrhage; (5) the absence of evidence for a relationship of the disease with epidemic hiccough (at variance with experience in Continental Europe); (6) the failure to discover predisposing causes save that those engaged in sedentary occupations seemed to be selected more particularly and that, as regards age incidence, the period 10-20 years proved to be most susceptible; (7) the frequency with which mild and atypical forms of the disease occur and are overlooked; and (8) the help for diagnosis that may be derived from examination of the cerebrospinal fluid.

The report includes a large amount of valuable statistical information as well as an extensive bibliography and a subject-index. Though intended chiefly to supply information to the medical profession in Great Britain, it will, as Sir George Newman suggests in his preface, make a much wider appeal, for it will be found to be a valuable work of reference by anyone who is interested in epidemic encephalitis.

L. F. B.